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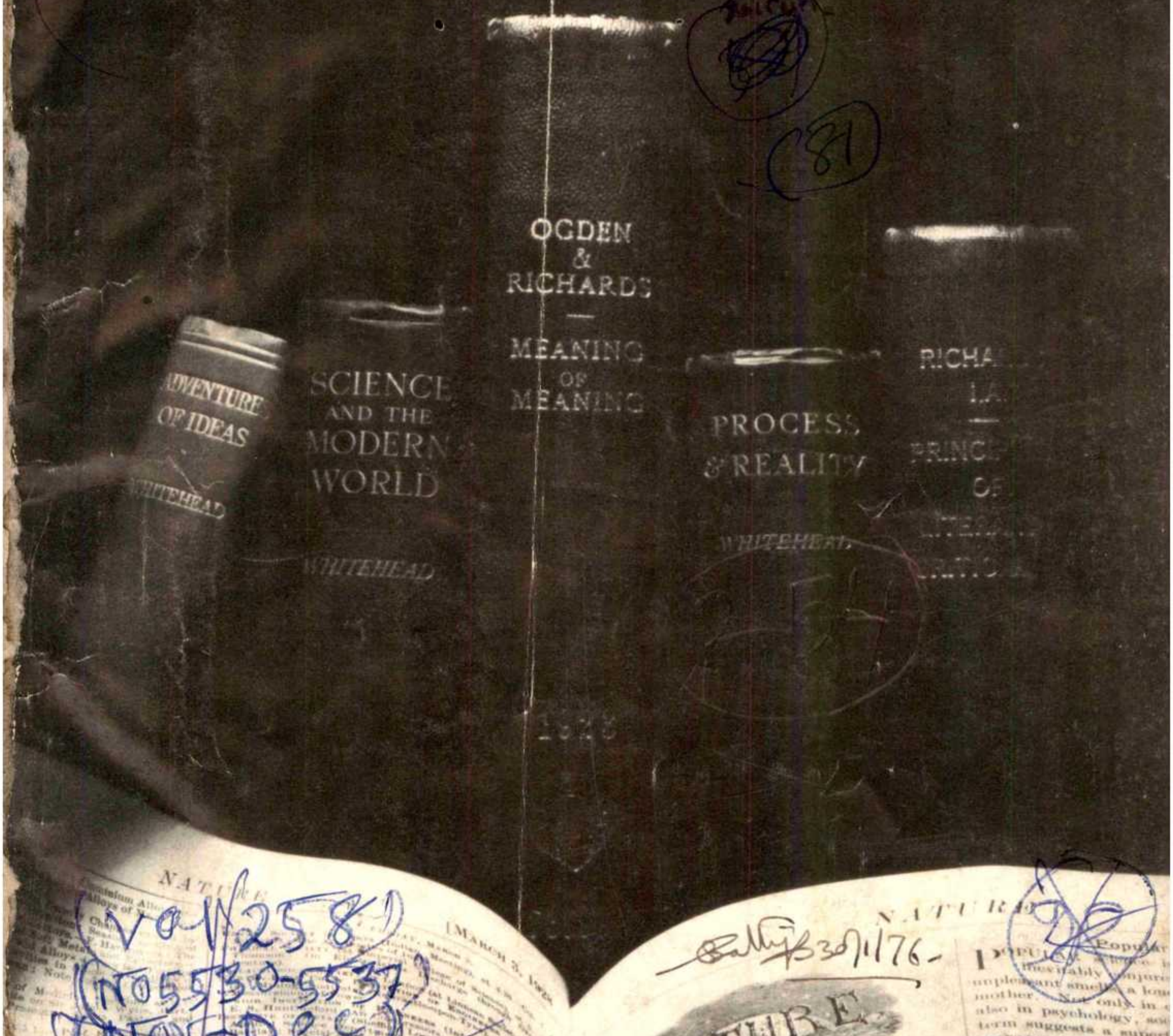

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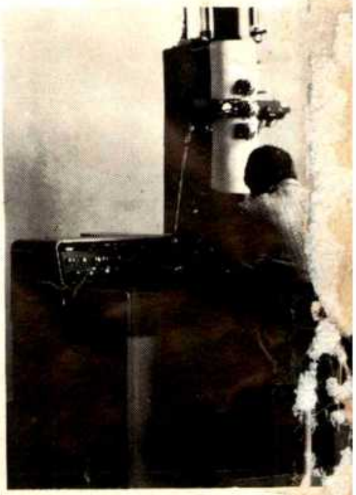

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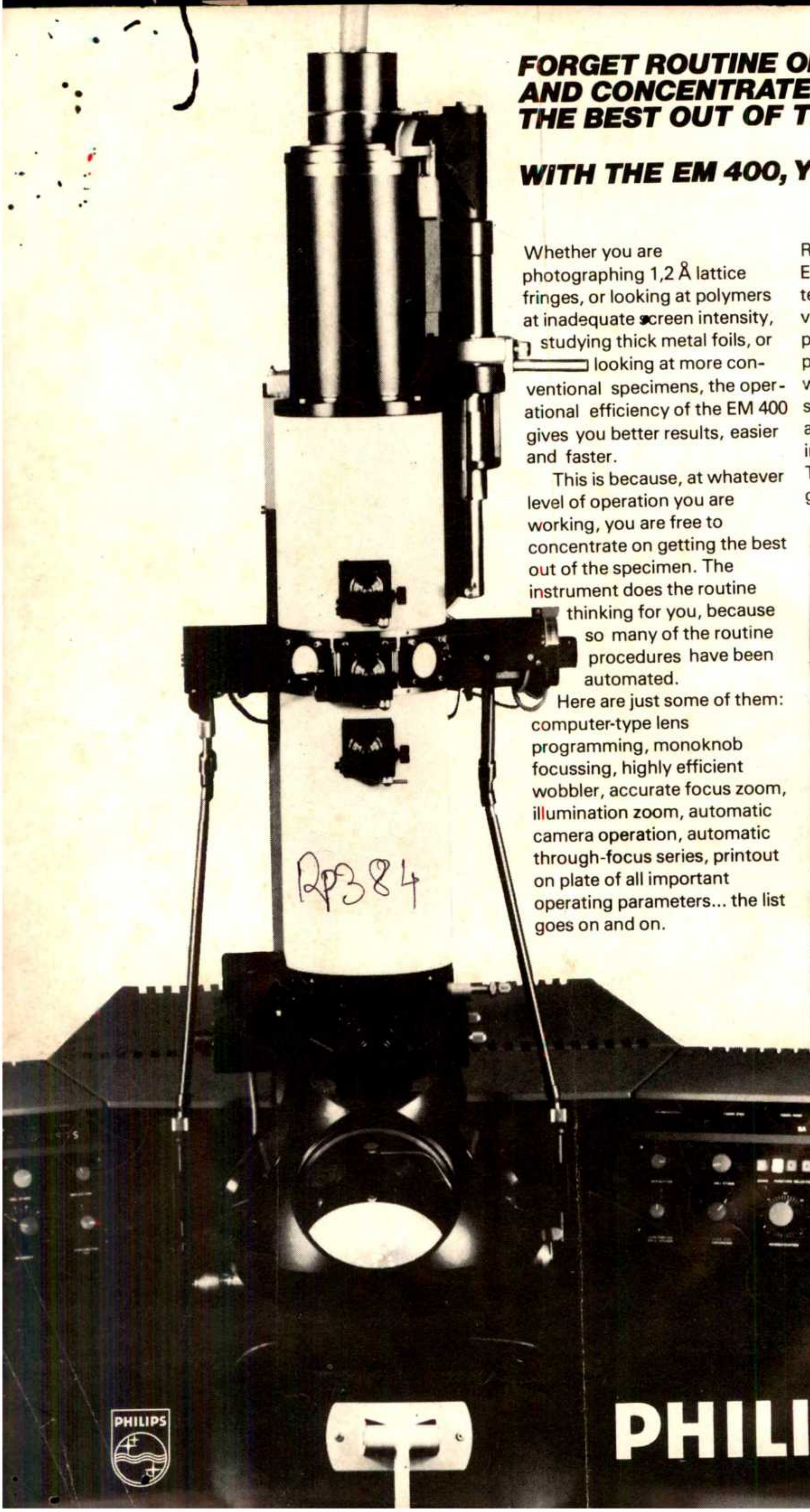

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Some of the books that the late C H Waddington and others were reading 50 years ago. See page 20. (By permission of the Royal Society. Photo: Michael Freeman.)

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At the end of last week we said goodbye to our News Editor of the past two years, John Hall, who goes to the *Daily Telegraph Magazine* as Assistant Features Editor. During his time at *Nature* he has, in particular, had a considerable influence on the design and layout of the journal. In his place we welcome Chris Sherwell, a journalist who joins us from the *Financial Times*.

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Weaver ants building their nest. For a discussion of how insects communicate by chemical signals, see pages 194 and 230. (Photo: NHPA.)

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Proceedings of the Florida Colloquium on Molecular Biology, March 13-14, 1975, sponsored by the Department of Biochemistry, University of Florida, Gainesville, Florida.

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(i) To refer to several references by the same author at once, only one reference number need be given.

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Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable: thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely. We are always glad to see artwork for possible use on the cover, whether or not it is associated with a manuscript, but cannot guarantee the return of material.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

A fuller guide appeared in *Nature* (**246**, 238; 1973).

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nature

November 6, 1975

Do scientific journals need a code of practice?

THE Scientific Information Committee of the Royal Society has recently put forward a set of guidelines for the refereeing of papers for publication. The status of the guidelines is unclear in the context in which they have been presented (as part of the programme for a conference of editors), but it seems that for the present they are open for discussion, and that even after such discussion they will only represent an informal set of rules to which editors may or may not choose to conform. We believe that such a voluntary code of practice is unworkable, will cause more trouble than it is worth and should be quietly dropped.

The committee is right in one respect: there is a growing feeling that the processes by which scientists regulate each other's activities, be it through grant-giving, refereeing and editing, or even control of membership of learned societies, could with profit be made somewhat more open. There are still, we think, strong objections to a system in which all is revealed and the names of referees and advisers are made public. But there are obvious intermediate positions in which the supplicant at least knows what sort of treatment to expect and can thus choose his own strategy accordingly. Unfortunately the issuing of guidelines suggests a move to uniformity, whereas what is actually needed is a preservation of diversity to allow individual scientists in individual cases to make individual choices.

Furthermore, once a set of guidelines is published there will be a tendency for some aggrieved authors to look to the guidelines for redress, even if the journal has never claimed to conform to them.

In matters of dispute it is clear that the drafting of the document leaves much to be desired, as loopholes abound. Three of the guidelines with which we disagree strongly are:

- Every paper submitted to a journal for publication should be refereed.
- No paper should be rejected on the adverse report of a single referee.
- A definite procedure should be established for editorial decision within a stated period.

However worthy these statements are, they are unworkable—indeed undesirable—in particular instances; and it is often in these very instances (admittedly rare) that the author is looking for a way to prolong the battle. What is one to do about the author who scatters his paper with unsubstantiated statements, demands that not one but two referees should see it and insists that the journal should decide within a stated period? Supporters of the guidelines say that this is where editorial discretion comes in and of course "every" doesn't mean "every". But this leaves us with an additional guideline—'the editor may choose to ignore these guidelines'. From a straw poll it is clear that many editors by no means conform and have no intention of doing so. It will surely bring the guidelines into discredit if they are widely ignored.

An alternative response to calls for more openness should be considered. If journals were to state regularly what their policy was in handling manuscripts, and the extent to which editorial discretion entered, prospective authors would actually have a clearer impression of how they stood.

With this in mind we have compiled the following brief statement of our editorial procedures.

Nature aims to publish scientific reports which are suited to a wider readership than a specialised journal can provide; manuscripts concerned with any branch of science will be considered. The spectrum of papers published broadly represents that of papers submitted.

All papers are assessed by one of the three manuscript editors (two, a biologist and a physical scientist, based in London; one, a biologist, based in Washington). These three editors may consult with other editorial staff and will return roughly a quarter of all manuscripts at an early stage, chiefly on the basis that they do not have any obvious broad appeal. The remainder are sent to one referee (occasionally two) and the referee's recommendation is generally followed. On rare occasions a negative report is overruled when there seems to be a pressing need for a particular paper to be made widely and rapidly available. Somewhat more often a paper that has received only a lukewarm recommendation from a referee is declined for reasons of limited space. Specific comments from a referee are usually passed on to authors.

The objective is to make a decision in principle on papers within a month of receipt, although authors are often asked at that stage to revise or shorten manuscripts. The present acceptance rate for submissions to *Nature* is around 35%, and following acceptance we aim to publish papers within six weeks.

We propose to publish an updated version annually.

Victory celebrations in Hanoi: pace of scientific organisation quickens

Vietnamese journey

Any country aspiring to an industrial and technological competence must have an underlying strength in basic science. The North Vietnamese know this, and taking their characteristic long view, have been organising their war-depleted scientific cadres into a versatile research nucleus. Their effort proceeded all through the war at whatever pace was possible, but wholesale evacuation of educational and research institutes to the countryside prevented rapid progress. As American participation in the war diminished and then ceased altogether, the pace of Vietnamese scientific organisation quickened. In June 1975, a scant six weeks after the formal end of hostilities, there were already strong indications that the Vietnamese will soon have high competence in the basic physical and biological sciences, as well as in the related applied fields of engineering, medicine and agriculture.—Arthur W. Galston reports.

AS a representative of the Scientists' Institute for Public Information (SIPI) and carrying a gift of books, chemicals and equipment from the Society for Social Responsibility in Science, I had been invited for a return visit to North Vietnam in June 1975. My first visit, made in April 1971 together with Ethan Signer of MIT, had led to interviews with Prime Minister Pham Van Dong, the Ministers of Higher Education, Public Health, and Culture, and visits to numerous scientific institutions. Now in a Vietnam freshly at peace, I had been invited to lecture in my speciality, plant physiology and biochemistry, to instruct young scientists in techniques of plant cell and tissue culture, and to initiate a scientific aid programme from the United States (through SIPI) to the new Vietnam.

About four miles north-west of Hanoi, a large, modern science research centre is nearing completion. Financed by the USSR and constructed under Soviet supervision with Vietnamese labour, the centre was originally planned and administered by the State Committee on Science and Technology, the leading scientific policy making group in Vietnam. Since the end of the war, and to insure rapid progress, the centre, under its newly appointed director, Nguyen an Hieu, has been placed directly under Prime Minister Pham Van Dong, to whom Hieu reports directly. At a private conference with these two men, I was struck by their close rapport, and by the Prime Minister's understanding of and direct concern with scientific matters. Almost unconsciously, I had to contrast this with the situation in the United States, where in spite of its vastly greater competence and importance, American science has had no science adviser close to the President since Richard Nixon dismantled the structure that had been created and successively strengthened by his five predecessors.

Hieu is a 39-year-old theoretical physicist who received his training under the noted Professor Bogolyubov at Dubna, the huge research centre near Moscow run by a consortium of Warsaw Pact nations. In addition to his role as director of the centre, Hieu directs the Institute of Physics, one of the six operating units of the centre. Since the completion of his PhD training in 1961, Hieu has made substantial contributions to the field of elementary particle theory, and now, as a member of the governing council at Dubna, visits that establishment every year. Five of his students have also received their basic training at Dubna, and in recognition of this growing Vietnamese competence in physics, the Dubna consortium recently donated advanced research equipment worth, conservatively, half a million dollars to the centre. This equipment is now largely set up and functioning in a series of huts and basement rooms of the centre, but with the imminent completion of that structure, will move into more spacious, even elegant, quarters.

Aware of the potential practical importance of research in solid state physics, Hieu has recently shifted to that field, and the laboratory's organisation reflects that emphasis. Already, large germanium and bismith-tellurium single crystals have been grown, their impurities removed in a diffusion separation furnace, and the resulting purer crystals thinly sectioned to furnish the elements of semiconductor diodes for amplifiers. Sprinkled around the laboratory is a mixture of advanced equipment from many nations in the Soviet bloc, as well as the latest in scientific publications from all over the world, efficiently and cheaply reproduced by the Chinese without royalty payments. I saw a large Soviet neutron generator in the activation analysis of minerals and proteins, several multichannel data analysers from East Germany, a computer of Hungarian design based on American integrated circuits, and an advanced spectroscopy laboratory with many components from France, Great Britain and West Germany. A large X-ray laboratory is involved in analysing the structure of crystals and a solar energy group is using semiconductors to translate light energy directly into usable quantities of electricity. These activities would be thoroughly familiar to western physicists, and it is clear that besides playing a role in the development of science in their own country, Vietnamese physicists are eager to enlarge their contacts with the rest of the world.

I could get no exact figures on the cost of the Science Research Centre but I judge its approximate area at

500,000 square feet of usable space. At even $20 a square foot, a ridiculously low level by western standards, the building alone will be worth $10 millions when completed. Together with its abundant scientific equipment, it should eventually be worth about $25 million. Though not especially impressive by American standards, this is enough to make it one of the largest installations in Vietnam. In the North, it will probably be second only to the $75 millions paper mill being donated and built by the Swedes under an official aid programme.

In addition to the Institute of Physics, the centre will house the Institute of Mathematics, founded in 1969 under Le Van Thiem, a German-trained PhD. The still embryonic institutes of biology, earth sciences and oceanography are as yet still without directors. A chemistry laboratory devoted to the study of catalysis will serve as a nucleus for the ultimate development of an Institute of Chemistry, for which the Vietnamese judge they do not yet have sufficient competent personnel. A computer centre will complete the presently contemplated facilities.

I was, in fact, the first visiting foreign scientist in what Hieu envisions as an expanding programme central to the intellectual life of the centre. My lectures on plant physiology and biochemistry were attended mainly by those biologists close to that area, but I was impressed with the fact that physicists attended when I spoke on effects of light on plants, and both animal and microbial biochemists attended when I talked on the biochemistry of plant growth hormones.

I instructed a small research group in some new biological techniques and found the young scientists well trained in basic concepts and eager to learn, though several years out of date. They were proud to be the occupants of the first three finished rooms in the entire centre. These had been completed much before schedule in connection with plans for my visit, and the Soviet engineer and architect came around during one working day to ask whether I found everything satisfactory. That struck me as meaningful *detente* in action.

Though the centre is the most competent of the research facilities in North Vietnam, it is certainly not the only one. Much work in strength of materials goes on at the Hanoi Polytechnic Institute, in vaccines at the National Institute of Hygiene and Epidemiology, in liver cancer at the Viet Duc Hospital, in natural plant products and pharmaceuticals at several institutes, and in agronomy and herbicides at the Agricultural University. By contrast, the University of Hanoi has pitifully few laboratories for modern research, despite its major educational role. The biologists there listened with obvious envy as the American programme of aid to the Science Research Centre was outlined. Couldn't we help them too, they wanted to know. But they understood that our present limited resources would make no impact at all if too widely disseminated. They felt somewhat better when I promised to seek specific aid for them from other American sources.

Though scientific groups were understandably eager to receive American aid, official or unofficial, there was general agreement that American scientists have a special responsibility to continue investigations on the yet largely unknown consequences of the massive military defoliation operations in the South. In the decade starting about 1971, we dropped from aeroplanes about one hundred million pounds of chemicals toxic to plants over an area of South Vietnam greater than that of the state of Connecticut. About a quarter of South Vietnam's dense upland forests, one-third of its coastal mangroves, and perhaps one-tenth of its agricultural lands, were sprayed in an attempt to deprive the guerillas of forest cover and food resources. In spite of several Department of Defense-sponsored studies by US scientists, the ultimate ecological effects of this massive operation are not yet known, but several immediate effects, especially in the mangroves, are known to be severe.

Perhaps more important is the danger to public health, for it is now known that one of the herbicides used, 2.4.5-T. contained significant quantities of an impurity called TCDD (2,3,7,8-tetrachlorodibenzodioxin). Very small quantities of this material, in the parts per thousand million range, can cause death, the malformation of developing embryos in laboratory rats and mice, and chromosomal aberrations in dividing plant and animal cells. The latter observation raises the possibility that TCDD can also cause mutations and cancer. Dr Ton That Tang is suspicious that the recent marked upsurge in liver cancer all over Vietnam may be related to TCDD persistence in the soils of sprayed areas. If this chemical were slowly released into streams, it would find its way into the algae and fish of the ocean, and ultimately into the human diet. Furthermore, since the ocean currents carry waters from south to north, Tung feels that TCDD sprayed in the South could affect civilians in the North. All this evidence is circumstantial, but since TCDD was detected in fish collected in 1973, two years after the herbicide spraying programme had been terminated, and since its persistence in soil and water is not known with certainty, the possibility of contained danger to public health and ecosystems continues.

Can the US turn its back on this problem? Or does American responsibility for investigation continue? Whether or not the US ever decides to give official scientific aid to Vietnam, many feel that it has a moral obligation to follow through on this research, much as it did after dropping an atomic bomb on Hiroshima.

Ta Quang Buu, a dynamic, Cambridge-educated mathematician, has served as Minister of Higher Education in North Vietnam since 1965. When I first met him in Hanoi in 1971, he impressed me with his keen, driving intelligence and his energetic devotion to the ideal of the best posible educational system for his country's youth, even in the midst of a debilitating war. But like many officials in the new Vietnam, Ta Quang Buu is finding the problems of peace more formidable than the problems of war.

Consider some of the difficulties facing him. One quarter of North Vietnam's population of almost 25 million is now at school. The figures for the South are not yet precisely known, but they are assumed to be rather similar. For the past 20 years, North and South have gone their separate educational

Plant physiologists at a Galston lecture

ways; their aspirations, philosophies of education and methodologies have been as different as communist and capitalist orientations can make them. Can two such systems possibly be unified? Should they be? If so, should change be gradual or sudden? What good points in the southern educational system can be adapted to the North? How should one handle the problems of adult education, given the imminent demobilisation of hundreds of thousands of soldiers? Although the end of the war came with dramatic suddenness, Buu's deputies have already travelled to the South for liaison with their counterparts. As staggering as the problems seem to be, Buu and his colleagues are tackling them with the same stubborn determination that characterised Vietnam's long military struggle.

In considering how best to mobilise and unify the educational and scientific resources of the North and South, Buu has analysed the situation in the South. Before 1973, he told me, university education in the South was completely dominated by the French model. The outstanding characteristics of this model are, first, an emphasis on general education, with only scant technical or vocational training being offered; second, the complete autonomy of the various faculties in devising standards, curricula and entrance requirements; third, no selective examinations at entrance, but rather massive elimination of students along the way (recently 60% have been eliminated after the first year and between 75 and 90% have never graduated); fourth, no assurance of a job after graduation and thus no limitation on the number of students choosing an option.

This unplanned system has resulted in an asymmetric and, according to Buu, chaotic expansion of the student population in southern universities. They now number more than 120 000, and of these, fully 50,000 are studying literature and law, preparatory to a legal career. "What can we do with so many lawyers?", exclaims Buu. "Ways will have to be found to alter the course of their studies, to divert them to other occupations". In similar fashion, the many students in training for financial careers will be retrained "to make good clerks and civil servants for the new system", and some of the many potential secondary school teachers will be encouraged to seek other occupations.

By contrast, the North Vietnamese system emphasises educational quotas in the various specialities as part of the planning for the filling of anticipated job vacancies. It also includes stiff, competitive entrance examinations, minimum dropouts along the way, and limited autonomy of the individual faculties under a more centralised and highly codified system of national education. Following this system, which Buu believes resembles the American more than the French, the North Vietnamese have admitted only 55,000 students to higher educational institutions. This represents a decline from the 70,000 students enrolled in 1973, caused by the raising of educational standards. Obviously, to integrate the northern and southern educational systems, some compromises must be made in standards for admission and dropout projections, as well as in educational objectives and practices.

Paradoxically, American educational innovations introduced into the South about 1973 have shaped the system more toward the northern model. For example, the introduction of two-year community colleges at Da Nang, Nha Trang, Tu Duc and several other locations accords well with plans in the North to reserve the established universities for the final year of more specialised training. Also, under American guidance, polytechnic institutes were established for the training of engineers and other specialists. This too fits in well with the North Vietnamese approach. Hanoi Polytechnic Institute, built during the war, with Russian aid, would seem familiar in basic organisation to a student at MIT or Caltech. With typical Vietnamese eclecticism, Buu proposes to combine the best aspects of these various systems into a new, indigenous approach. "Bilateral accommodation" and "smooth interpenetration" were some of the phrases he used to describe how the process might work.

Even before the final formula has been devised, educational institutions closed by the war have been reopened. For example, Hue University was back in operation by April 14, and the Medical and Pharmacy faculties in Saigon reopened in June, barely a month after the end of the war. Though a few teachers fled with the departing South Vietnamese officials, almost all, according to Buu, are fitting in well with the new system, and are actively seeking guidance in making the accommodation. This is especially important since the student body, previously limited to the privileged classes, will now include many peasants and workers, for whom new educational techniques will have to be introduced.

Buu believes that equality of educational opportunity has already been widely implemented in the North, through improvement of rural secondary schools, through extensive adult education programmes, and by giving preference in university admissions to those with some work or military service experience. This year, approximately 35–40% of the 10,000 students to be admitted to universities in the North will have such work experience. Since there will be about 80,000 applications in all, this will give a substantial competitive advantages to the more experienced applicants. Yet, Buu is all for flexibility, and definitely rejects the Chinese model of requiring work experience before admission to the university. Especially gifted or critically needed students should not, he feels, be held to that requirement.

There are as yet no postgraduate training centres in Vietnam for work leading to the PhD or equivalent degree. During the war, it was the practice to send promising students abroad, especially to the Socialist countries of eastern Europe, for such training. In 1969, there were about 12,000 such students abroad; the number is now down to about 7,000 and Buu expects it to go even lower as centres for such training develop in the time when some promising candidates can be sent to western Europe Vietnam. But he also looks forward to and even the United States, especially for training in science and technology.

Can the United States play a role in the postwar educational reconstruction of Vietnam? Like Prime Minister Pham Van Dong, noted surgeon Ton That Tung and other officials I interviewed, Buu insists that the Vietnamese do not hate, and in fact in many ways admire Americans. Under proper conditions, he would be glad to receive American aid. Gifts of books and scientific equipment, lectures and seminars by visiting specialists and exchange by communication are possible and desirable immediately. Exchanges of teachers and students are not desirable or possible now, but may be within two or three years, after the reunification of North and South has been accomplished, and their major educational patterns have been firmly charted.

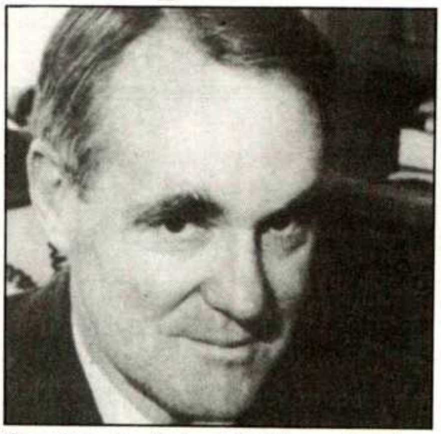
Since American science and technology has been so extensively used for destructive purposes all over Vietnam, Buu believes it would be uniquely appropriate for groups of scientists to lend their talents to the reconstruction efforts now under way. In the light of President Ford's and Henry Kissinger's stated opposition to any such official aid, assistance would have to be unofficial and "people to people". The joint project of the Scientists' Institute for Public Information and the Society for Social Responsibility in Science was, he felt, an appropriate model. But he is neither doctrinaire nor proud in this matter. He will accept any appropriate aid given in good faith, confident that the future will bring a normalisation of relations between his country and the United States. "Then", he says, his eyes lighting up, "the vast wealth and power of the United States can really be focused on the job of rebuilding our underdeveloped and battered country". ●

Cornforth's prize

J. W. CORNFORTH, who shares this year's Nobel Prize in Chemistry with V. Prelog, is now taking up, at the age of 58, a new full-time appointment as Royal Society Research Professor in the Department of Applied Science in the University of Sussex. He began his distinguished career in organic chemical research in Australia: he graduated at the University of Sydney and was awarded the University Medal in Organic Chemistry. This was followed by an 1851 Exhibition Overseas Scholarship to the UK, which he held from 1939 to 1942 in the Dyson Perrins Laboratory at Oxford with Robert Robinson, and he has remained in the UK since.

His early work was on Australian plant materials (with J. C. Earl) and he moved in Robinson's laboratory naturally. Inevitably in that environment he was attracted to the problem of the laboratory synthesis of cholesterol, and when the Second World War extended his stay in the UK, he was drawn into the maelstrom of the penicillin problem. In 1946 he joined the staff of the Medical Research Council at the National Institute for Medical Research and a career of individual work began to take shape; a summary is almost impossible to make: it would be a task of selecting highlights in a dazzling array of subjects. Enquiry into natural products, laboratory synthetical methods, fundamental reactions of biosynthesis, isotope techniques, reactions applicable to manufacture of medicinal products—all were grist to his mill; one has only to start discussion of a structural or synthetic problem with him to provoke a flow of 'graphite and cellulose' solutions. The only drawback to consultation is his complete deafness, which makes the problem of getting a word in edgeways and saying "yes, but" or "I thought of that" a difficult one. The handicap of deafness he has overcome magnificently, particularly with the aid of his wife, Rita, who is herself an organic chemist of great skill.

Cornforth's relation with Robert Robinson is best put in his own words.



Opening the Robert Robinson Lecture he gave to the Chemical Society in 1972 he said: "Our friendship . . . for more than 30 years has been a continuous sequence of differences of opinion". Reorientation of Cornforth's work, after the publication of some papers on miscellaneous subjects from the National Institute for Medical Research, came about in two ways in 1950. First, as a consequence of the Medical Research Council taking the lead in this country in the search for a source from which cortisone could be manufactured, and its involvement with the National Research Development Corporation, the workers at the National Institute were mobilised. Hecogenin, the steroid sapogenin from sisal (*Agave sisalana*) was found to be such a raw material, which, on the basis of fundamental chemical work at Mill Hill and development in collaboration with the staff of Glaxo Laboratories became of considerable commercial importance.

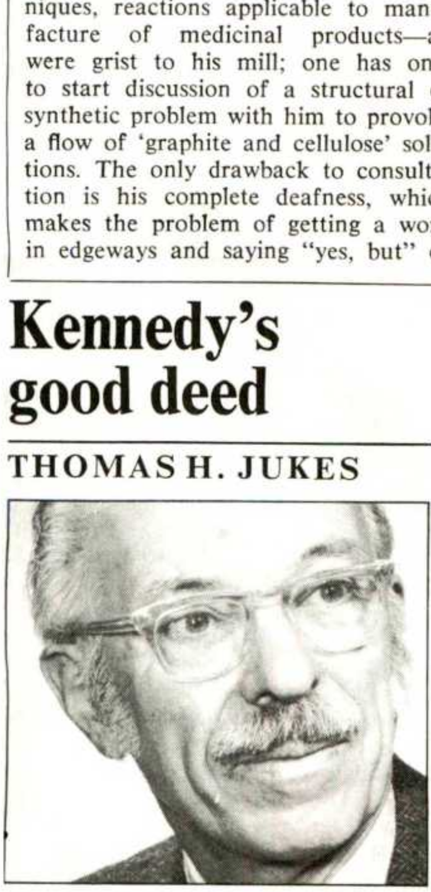
The second direction of development of Cornforth's work came in the early 1950s in association with George Popják, then in the Biochemistry Division at Mill Hill and later at the Medical Research Council's Radiopathology Research Unit. This work, which led, finally, to the results of fundamental theoretical significance for enzymology that have earned the Nobel Award, began with the examination of the problem of the route of biosynthesis of squalene from mevalonic acid. Development of new techniques for using isotopically labelled compounds, with such incidental ingenuities as the characterisation and orientation of the three hydrogen atoms of a methyl group by labelling with protium, tritium and deuterium followed, along with the extension to the study of enzyme stereochemistry on a wider basis.

In 1962, Cornforth and Popják became joint directors of the Shell Research Milstead Laboratory of Chemical Enzymology at Sittingbourne, an establishment that in the best tradition of schoolboy humour was dubbed at its inauguration the "Pop-Corn Laboratory". Unhappily Popják broke the link when he migrated to the USA. No doubt Cornforth's new laboratory in the University of Sussex will acquire some title of affection appropriate to the unique individuality of its presiding genius and certainly occupy an important place among the world's centres of scientific research.

R. K. Callow

Kennedy's good deed

THOMAS H. JUKES



ON September 8, the US Senate passed, by 61 votes to 29, a bill (S-963) introduced by Senator Kennedy to ban the use of diethylstilboestrol (DES) in growing beef cattle. The same bill would authorise the use of DES as a contraceptive after sexual intercourse only in rape, incest or "a comparable medical emergency" (the 'morning-after pill').

The bill carefully provides that the ban for beef must first be approved by the National Cancer Institute. This will be easy. Scientists of the National Cancer Institute have already testified before congressional hearings that one molecule of DES may cause cancer. Quite a lot of cancer cases were thus implied in a world population of 3×10^9 which is presumably exposed to molecular levels of DES in the air and water. One morning-after pill contains 7×10^{19} molecules.

Diethylstilboestrol is a known human carcinogen. Even worse, it is synthetic and man-made; a self-inflicted wound. Herbst, who discovered its carcinogenicity in human subjects, has calculated its cancer-causing potency: less than 4 cases per 1,000 in the female progeny of pregnant women receiving 1.5 mg (3×10^{18} molecules) of DES per day. He also notes that "there has been no documented correlation of maternal DES therapy with the development of cancer in males". Incidentally, DES is widely used as an anti-cancer drug in prostatic cases.

The intake of DES per capita from beef may be calculated from data for the residues found in implanted cattle, and the consumption of beef liver, 0.7 kg a year (0.12 parts per 10^9), plus beef muscle meats, 52 kg a year (0.012

parts per 10^9), in the USA. The intake corresponds to a daily dose of 1.9 ng, which is about 1.3×10^{-6} of the 1.5 mg used in Herbst's calculations. On a linear basis, the cancer risk to female offspring is, therefore, less than 5 cases per 10^9, corresponding to less than one case per 133 years in the 1.5 million females born annually in the USA. How much less than one case?

Probably quite a bit, but actually unmeasurable in view of the fact that other oestrogens predominate over this level the efficiency of conversion of feed into DES. Nubile women secrete oestrogenic potency equivalent to 300,000 ng of DES daily, and wheat germ contains oestrogens, measured by ingestion, equivalent to about 400 ng of DES per gram. Also, DES in beef liver is said to be present as glucuronide, which is inactive. The organic food crowd will applaud Senator Kennedy. More important, they will vote for him. Consumers will pick up the tab for the extra cost of beef production occasioned by the ban, which will decrease the efficiency of conversion of feed into lean meat by about 10%. Food prices are increasing so rapidly that this extra cost of up to $900 million will be lost in the spiral.

Of course the money might have been used for cancer prevention and treatment . . . But who wants to take a chance on getting cancer, even one case in 133 years in 200 million people of a form of cancer that responds to treatment?

Banning DES will call for about 7 million tons of corn to maintain the US beef production at its present level. Senator Kennedy has professed sympathy for the plight of starving people in Bangladesh. Seven million tons is a lot of corn. But sympathy has to make sense. These people don't vote in Massachusetts!

international news

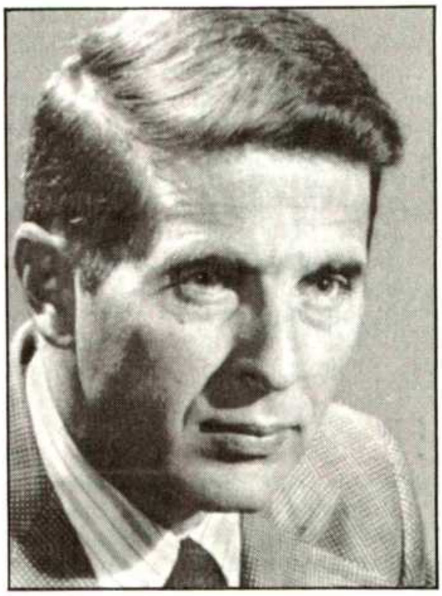
Computing the chances of a nuclear accident

by Colin Norman, Washington

FOR years, spokesmen for the nuclear industry and the federal government have been assuring the American public that the risks associated with nuclear power plants are vanishingly small. The nuclear industry has a safety record unblemished by any accident which has released radioactivity into the environment, the argument goes, and nuclear power plants are bristling with so many safety devices that the chance of something going calamitously wrong is close to negligible.

But, as five years of raging debate have amply demonstrated, those bland assurances have not convinced everybody. So, in August 1972, the Atomic Energy Commission decided—though with some doubts about the feasibility of the project—to launch a mammoth computer analysis of the probability that a nuclear reactor could go haywire and endanger the lives of people nearby. The final results of the analysis, which were made public last week, support the official assurances. Nevertheless, they are unlikely to still the nuclear debate.

In general terms, the analysis suggests that there is a finite chance of a catastrophic nuclear accident, but the risk is miniscule when compared with other hazards such as fires, explosions, earthquakes, tornadoes or hurricanes. As William A. Anders, Chairman of the Nuclear Regulatory Commission (NRC)—which assumed responsibility for the project when the Atomic Energy Commission was dismantled earlier this year—suggested last week, the analysis "reinforces the commission's belief that a nuclear power plant designed, constructed and operated in accordance with NRC's comprehensive regulatory requirements provides protection to public health and safety and the environment".


William Anders: reassuring

Carried out under the direction of Professor Norman Rasmussen, a nuclear engineer at MIT, the study involved a technique, widely used in the defence and aerospace industries, known as "event tree analysis". In short, the technique attempts to predict the chances of failure of various components in a reactor and to assess the effects in terms of release of radioactivity, loss of lives and damage to property. The Rasmussen study explored thousands of potential sequences of failures in reaching its conclusions.

The final results, contained in a massive 12-volume report, conform closely to preliminary estimates published by Rasmussen in a draft report last year. Although the draft report attracted more than 1,800 pages of comment from various individuals and groups—much of it highly critical—the most significant change required in the analysis, according to Rasmussen, was a large increase in the estimate of the number of cases of cancer resulting from a massive release of radioactivity.

The study concentrated on the possibility of occurrence of a string of mishaps which would cause the core of a reactor to melt. Surprisingly, the analysis suggests that the chances of a core melt are about 1 in 20,000 per reactor year, which is rather higher than many previously quoted estimates (which have generally been put at about 1 in 10^6). But it suggests that the consequences of such an accident may not be catastrophic. "Contrary to commonly held belief that . . . core melting would surely result in severe accidents with large consequences", the report states, "the magnitude of the potential consequences of a core melt accident were found to have a wide range of values. The probability is high that the consequences would be modest".

The analysis suggests, in brief, that if a core melts, the pressure vessel which encloses it would not necessarily rupture and release large quantities of radioactivity into the atmosphere. The core would, however, eventually melt its way into the ground, but the study suggests that most of the radioactivity would be trapped in the soil. The most likely consequence of a core melt, the study predicts, would be less than one death and less than $1 million worth of damage to property outside the plant itself.

The worst possible case would involve a series of accidents leading to a core melt, followed by a massive explosion which ruptures the pressure vessel and releases large amounts of radioactive gases into the atmosphere, a steady wind blowing toward a populous area, and rain occurring at a critical time so that most of the radioactivity would be dumped on the population centre. The possibility of such a calamity would be about 1 in 10^9 per reactor year, the analysis suggests, but it would result in about 3,300 deaths and cause about $14,000 million worth of damage to property.

The results of the analysis are stated in many ingenious ways in the report, the most widely quoted of which is sure to be the statement that a person living near a reactor stands a 2,000 times greater chance of being killed by lightning than of being killed by a nuclear accident.

Rasmussen said last week that he has high confidence in the accuracy of the predictions, and he pointed out that the methods used in the analysis have been endorsed by many groups. He readily acknowledged, however, that the study has its limitations. It does not deal with the possibility of sabotage, for example, but Rasmussen said at a press conference that it would be difficult to cause a very severe catastrophe by sabotage because of the existence of safety devices to prevent large releases of radioactivity following a core melt.

The analysis, moreover, is limited exclusively to an accident at an operating reactor, and does not include an assessment of the risks associated with waste disposal, the potential theft of atomic bomb ingredients by terrorist organisations, or other frequently cited hazards of nuclear power. The focus of the anti-nuclear power movement has recently shifted to those areas.

Needless to say, Rasmussen's conclusions have not met with widespread endorsement from groups which have been critical of the nuclear power programme in the past. Dr Arthur Tamplin, a scientist on the staff of the Natural Resources Defense Council, last week suggested that the report has little application to the real world. "Those numbers are fine for an academic discussion in nuclear engineering departments", he said, but "the exercise has no reality". In particular, he questioned the study's predictions about what would happen after a core melt, suggesting that the behaviour of a mass of molten fuel is entirely unknown. Another critic was even as unchivalrous as to suggest that the report was a typical computer programmer's nightmare of "garbage in and garbage out".

Nevertheless, the study is the most ambitious attempt so far to place hard numbers on the official assertions that nuclear power is safe, and it will clearly form the basis of nuclear regulation in the United States for many years. Rasmussen warned, however, that the results of the analysis should not be extrapolated beyond the first 100 or so operating reactors in the United States. Later reactors, he suggested, will be even safer because of constant improvements in the safety systems.

As a footnote, it should be noted that the report could actually be seen as a boon for critics of nuclear power. At present, insurance against nuclear accidents is limited by law to a total of $560 million per accident. The law, known as the Price–Anderson Act, has been bitterly contested by nuclear critics, who argue that in the event of a nuclear accident, full compensation should be paid for health and property damage. If the dangers are really as remote as the Rasmussen analysis predicts, and the consequences likely to be small, then why should there be any limits to the liability? A statement published last week by the Friends of the Earth suggested that the Price-Anderson Act "points out the hypocrisy and inconsistency of the federal government's policy towards nuclear power". □

FOR the first time since 1969, the number of students enrolled in postgraduate science and engineering courses in the United States increased last year. According to a survey carried out by the National Science Foundation (NSF), the increase amounted to nearly 6% and the total number climbed back to the level of 1967.

Although the upturn makes a welcome change for many universities, which have been financially hard pressed and which have had to impose a virtual freeze on faculty recruitment partly because of declining enrolments in the early 1970s, the renewed interest in postgraduate education is of uncertain durability and it is concentrated heavily in a few fields.

The sharpest increase occurred in the life sciences, where the number of postgraduate students jumped by 11.3%. Psychology and the social sciences also showed substantial increases—8.0 and 6.6% respectively—while in engineering and the physical sciences there was only a slight upward trend. Engineering enrolments increased by 3.3% and those in the physical sciences by 1.4%; enrolment for the mathematical sciences declined a little.

The increases are surprising since they came at a time of continuing decline in direct government support of postgraduate students. The number of postgraduates receiving support from the federal government has declined by some 42% since 1967, and the trend shows little sign of abating because it is declared federal policy to phase out most graduate fellowship programmes. In contrast, the number of postgraduates supporting themselves through their studies is increasing—numbers were up by 14% between 1973 and 1974.

One explanation for the renewed interest in postgraduate education is that many scientists and engineers who received their first degrees in 1973 were faced with a tight job market, and elected to stay on at the universities to increase their chances of finding suitable employment. As far as enrolment in the life sciences is concerned, the sharp increase probably reflects the fact that there is at present a fashion in the United States for studying medicine—a year or so ago it was law—and many students who failed to get into medical schools turned to closely related fields in the life sciences.

Does the upswing in graduate enrolments indicate a return to the golden years of university expansion in the 1960s? Almost certainly not. The driving force for expansion a decade ago arose partly from large increases in federal support for science and technology and partly from the fact that the post-war baby boom was passing through the universities. The federal science budget is now virtually stagnant in terms of spending power, and demographic trends suggest that there will be little expansion of the college-age population for the next two decades.

One particularly interesting fact to emerge from the survey is that the number of women enrolled in postgraduate science and engineering courses has been increasing sharply during the past few years. Women represented 24% of all full-time graduate students last year, and their numbers had increased by 13% since 1973. They were concentrated chiefly in the life sciences and the social sciences. The survey also reflects the fact that government restrictions on the employment of foreign students is continuing to depress their numbers. There were 3% fewer foreign students enrolled in postgraduate science and engineering courses last year, and their representation in the total postgraduate population declined to 16%.

Brazil and biodegradable detergents

from Bruce Handler, Rio de Janeiro

THE Brazilian Congress is studying a proposal to ban the manufacture of non-biodegradable detergents in this country, which is slowly waking up to the problems of pollution. The bill, which orders detergent makers to produce only non-polluting products, has passed the House Constitution and Justice Committee. It still must win the approval of the entire House, the Senate and the President to become law, however. Observers in Brasilia say the chances are good, in view of other recent indications that Brazil is abandoning its one-time policy of development at any price in favour of one that shows more concern for protection of the environment.

The detergent bill came as a result of a recent invasion of foul-smelling white foam that drifted off the Tiete River in the south-eastern part of the country and literally covered the Sao Paulo suburb of Santana do Parnaiba. The tropical town looked as if it had been hit by a freak snowstorm. Environmental experts were unanimous in blaming excess amounts of non-biodegradable detergents that had been dumped into the river as the principal cause.

Concern over the Santana do Parnaiba incident was expressed as far away as Argentina, because the polluted Tiete empties into the Parana–Plate river basin. Because of Brazil's relative lack of concern about pollution until now, it may be prohibitively expensive to implement the detergent bill, even if it is passed quickly. Brazil at present imports all the benzine distillates needed to make biodegradable detergents, and because of a disastrous balance-of-payments situation caused by the high price of foreign crude oil, the government has ordered a drastic cutback on all but the most essential imports. The situation is even more pessimistic when one takes into account the fact that detergents are responsible for only a small percentage of water pollution in Brazil.

In August, President Ernesto Geisel signed a decree saying that all industries in Brazil must control pollution and giving the federal government the power summarily to close factories that foul the envinronment. This is quite a change from Brazil's position on pollution just a few years ago, when it told an international conference on the environment: "A country that has not yet reached satisfactory living standards cannot sidetrack its resources for pollution control."

The new decree does not mean, however, that this fast industrialising nation of 110 million people is going to become a paragon of environmental protection overnight. In fact, many pollution-conscious people here felt the immediate purpose of Geisel's decree was to stop state and local government from cracking down too hard and too fast on polluters, and thereby interfering with national development plans.

Cabinet ministers who suggested the anti-pollution measure to the president noted that although Brazil has an "acute" industrial pollution problem, the government must be careful "not to disorganise productive activities or cause social unrest" This seemed to be fancy wording for the problem of citizens in several Brazilian cities who have shown themselves to be at their wits' end because of pollution—and who would take to the streets in protest, if public demonstrations were not illegal under the military-controlled regime here.

A typical case occurred recently in the industrial town of Contagem outside Belo Horizonte, Brazil's third largest city. There, a cement factory whose smokestacks spew out over 100 tons of particles a day on local residents' houses had been promising for years to install pollution control equipment but never did. The people of Contagem complained that the factory was responsible not only for the white dust that covered everything in sight but also for widespread respiratory ailments.

Mayor Newton Cardoso finally ordered the factory closed. He said that "the human being must always be the focus of the government's attention." State police were sent out to make sure the plant's executives began shutting down production equipment, to comply with a deadline the mayor had set. In addition, the DOPS—the much-feared political police—went to the cement factory after hearing reports that townspeople were going to hold a demonstration there to celebrate Mayor Cardoso's decision.

Then the presidential decree came out. A judge rescinded the mayor's order, on the grounds that local authorities could no longer single-handedly shut down factories. The cement plant continues to operate, although its owners have promised to install anti-air pollution devices in 1976.

Paulo Nogueira Neto, who holds the recently created federal post of special secretary for the environment in Brazil, summed up the dilemma: "The important thing is not to pollute. But we also cannot ignore the economic side of the problem." He added that the new presidential decree "makes it clear that pollution control is obligatory and that there will be no loosening up."

Brazil has made remarkable economic progress since a development-minded but politically repressive government took power in 1964. But it has paid a high price in ecological terms.

Pollution in Sao Paulo, the nation's largest city, has become so bad that a leading department store now stocks anti-pollution masks—in adults' and children's sizes. Sao Paulo's last mayor won applause from the populace when he temporarily closed a polluting cement factory there. But this led to a political fight with the governor, and the mayor lost his job. The factory is open again, and the particle fallout is as bad as ever.

Local authorities in the southern state of Rio Grande do Sul succeeded in closing down a foul-smelling cellulose factory that had been built by a big Norwegian pulp and paper manufacturer. But that factory is now in business again. The Norwegians sold their share of the undertaking to private Brazilian interests, and an influential retired Army general was installed as president of the company.

Federal Representative Jose Roberto Faria Lima, a supporter of the bill to ban non-biodegradable detergents, made this comment: "It's too bad the Tiete River had to become covered with foam before the need was shown for a law requiring the production of biodegradable detergents in this country. Anti-pollution plans and even the President's decree don't do any good. Simply saying: 'It's against the law to pollute' doesn't stop pollution". □

Coal standard

from Vera Rich

DONETSK, in the Ukrainian SSR, has been accepted as the standard coalfield by the Eighth International Congress of Stratigraphic and Carboniferous Geology, which met recently in Moscow. The phenomena of the Donetsk field will be used as a basis for a unified international scale to be used in prospecting and establishing the boundaries of coal fields. The scale, which was proposed by a team of geologists from Britain, France, the USA and the Soviet Union, is at present at the draft stage. Nevertheless, the acceptance of Donetsk as the standard seems unlikely to be changed by further discussions, since this field is unique in giving a clear and detailed picture of the history of the whole of the Carboniferous period (345–280 Myr BP) Although Donetsk was selected on the basis of its geology, this coalfield has a history which may serve as a promising augury for international cooperation. As its original name of Uzhovka indicates, it was first opened up (1869–1870) by a Welsh mining expert by the name of Hughes. □

Letter from Israel

from Nechemia Meyers

AN idea first put forward by Zionist visionary Theodore Herzl 73 years ago, and then brought up again four decades later by an American soil conservationist, Walter Clay Lowdermilk, may soon, at long last, be implemented.

Herzl, Lowdermilk and others have advocated the construction of a canal (or tunnel) through which seawater would flow from the Mediterranean to the Dead Sea so that the difference in level of 400 m could be exploited for electricity generation.

Advocates came and went, but only recently, prompted by the energy crisis, did the government appoint a committee seriously to consider the possible implementation of such a scheme. The committee, headed by Bar-Ilan Professor Shlomo Eckstein, had before it a plan for building a 75-km tunnel which would bring Mediterranean water to a reservoir in the Judean Desert highlands overlooking the Dead Sea, from which it would be 'dropped' through turbines with an installed capacity of 300 MW.

The next stage, if the Eckstein committee's recommendations are accepted by the government, will be a 'prefeasibility study', including test drillings at various places along the proposed route.

According to present estimates, the project will require 10 years to complete and cost more than $200 million (at present prices). In operation, it will not only increase electricity supplies but also replenish the rapidly evaporating waters of the Dead Sea, from which large quantities of potash, bromine and other valuable minerals are extracted Traditional sources of replenishment, particularly the inflow from the Jordan River, have gradually dried up as more and more sweet water is being syphoned off upstream by Israelis and Jordanians for irrigation purposes.

There is, however, a limit to the amount of seawater that can be poured into the Dead Sea if it is not to overflow. Thus the turbines can only be activated for some 2,700 hours a year. As conceived at present, the flow would be scheduled to coincide with periods of peak demands for electricity, when hydroelectric power would supply 10% of Israel's estimated electricity requirements (in 10 years' time). Should other power stations be put temporarily out of action, however, the turbines could be kept in operation for an extended period, after which they would have to be shut down for two or three years while the Dead Sea 'dried up'.

The substantial quantities of water flowing through the proposed tunnel, planners say, could also be used for cooling purposes by nuclear power stations, making it possible to build them away from the crowded seashore.

● Israel does not yet have a nuclear power station on the coast—or anywhere else for that matter—but every 'nuclear development' in the country arouses interest, often for the wrong reasons. This was demonstrated once again a few weeks ago when a Dutch newspaper printed a photograph of a tower-like object and captioned it as follows: "A new nuclear energy installation is being completed near Tel Aviv. No details about this installation at the Weizmann Institute are known. However, the great powers are not only interested, but also worried about Israel's growing atomic strength."

In point of fact, the object shown is the 19-storey Koffler Accelerator Tower, next year to house a 14-UD Pelletron accelerator.

Far from being a secret facility, the Pelletron will be open to scientists from around the world. Indeed the first large international group to visit the site—participants in the recent Third Tandem Accelerator Conference—were there at the same time the Dutch newspaper was making its mistake.

● While Israeli physicists concern themselves, for the most part, with basic research, Israelis in the biomedical field generally concentrate on the solution of day-to-day problems. Such is the case with Professor Bruno Lunenfeld, Director of the Institute of Endocrinology at the Sheba Medical Centre, near Tel Aviv. Professor Lunenfeld, for example, has developed a method that enables previously childless Orthodox Jewish women to have children without violating the tenets of family purity as laid down by traditional Judaism.

For an Orthdox woman, sexual intercourse is only permitted during a limited period each month. It can begin only seven days after the end of her menstrual period, until when she is regarded as 'unclean'. In ordinary circumstances, this does not prevent her from becoming pregnant, but if a woman's menstrual period extends beyond the usual five days, or her menstrual cycle is less than the usual 28 days, her days of greatest fertility may occur during the time when she is 'unclean' and therefore forbidden to her husband.

Professor Lunenfeld found that this problem can almost always be solved, once the religious woman's most fertile days are determined, by giving her an anti-oestrogenic substance known as clomiphene citrate. As a result, ovulation is delayed long enough to occur when the woman is permitted by the laws of family purity to have intercourse. Ironically, this substance was originally developed as a means of birth control, but is now used primarily to induce ovulation in infertile women.

Incidentally, Moslem women have also benefited from the treatment. Subject to even more severe laws of purity than are their Jewish sisters, the anti-oestrogenic substance has saved many of them from childlessness, a particularly tragic fate in traditional Moslem society.

● In another part of the Sheba Medical Centre, Dr Dan de-Medina is using the techniques of biofeedback to treat veterans of the Yom Kippur War.

Dr de-Medina, whose professional career began with a cancer research team and later continued in the sphere of memory studies, first delved into biofeedback when on an extended visit to the USA in 1973. He arrived back in this country, fortuitously, shortly before the outbreak of the Yom Kippur War, in the wake of which hundreds of men suffered from post-traumatic neuroses. And in spite of the fact that biofeedback techniques had never previously been used in Israel for the treatment of such neuroses, Dr de-Medina was allowed to use them with some of the badly shaken war veterans.

He found, as did others dealing with this problem, that it was more common and more severe than it had been after previous wars, presumably because the fighting had lasted longer and because the outcome had been less clear-cut (the euphoria of a great victory apparently has a healing effect). The patients sent by the army to Dr de-Medina generally returned to relative normality more quickly than those treated by other techniques, some of which, unlike biofeedback, involve the possible creation of drug dependence.

The method used by Dr de-Medina (and overseas practitioners) is quite simple It involves connecting up the patient to a 'Galvanic Skin Response Feedback' instrument, not unlike a lie detector, which registers anxiety on a scale or by the sounding of a siren. After a period of conditioning, usually involving some 20 sessions, the patient finds he is able by his own efforts to reduce the number registered on the scale or the pitch of the siren and, almost as a side effect, to lessen his sense of anxiety Finally, he gains a conditioned reflex whereby anxiety is switched off, without the benefit of the instrument.

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correspondence

Industry and Academics

Sir,—I would like to comment on your leading article "Why industry and academics go their separate ways" (September 25).

Whereas in the large industrial countries, in spite of the problems cited in the article, there is probably always enough dialogue between research and industry to produce good results, in small countries like Norway scientific effort has to be very carefully husbanded. They cannot afford the luxury of *laissez faire* in research and development, and somehow they have to overcome the pre-development, evaluation and identification gaps, even if they don't satisfactorily solve them.

I have been living for much of the past 12 years in Norway, working in, or in the closest vicinity of, an interesting semi-academic institution which represents one solution to the problem presented in your article. This institution has been in existence for 25 years now, is still flourishing, and is therefore perhaps worthy of mention. It consists of a contract organisation by the name of SINTEF, situated on the campus of the Norwegian Technical University. It has a staff of some 650 people, of whom 350 are university graduates or the equivalent. In that it works hand-in-glove with the university, it is instrumental in keeping the latter in close touch with industrial trends, and in that most of its income is derived from industrial projects, it is unable itself to stray too far from industrial needs.

SINTEF is a non-profit-distributing organisation, with a board consisting of academics and industrialists. Money from the National Research Council is obtained largely according to the project in hand, and often on a 50–50 basis with an industrial partner Good ideas generated internally are backed internally until ripe enough for presentation to industry, at which point they are then, in the ideal situation, developed into a marketable product at industry's expense Good ideas, or requirements, from industry are brought to SINTEF for fundamental development because the latter has by now built up a solid reputation for quality, timeliness and (believe it or not) budget consciousness. A fairly thorough presentation of SINTEF will be published shortly in the *European Journal of Engineering Education.*

I often ask myself why this tripartite symbiosis actually works, and I think there are at least three good reasons. First, the university is not isolated from industry as so many are in larger countries. Second, a substantial number of research-oriented people get a great kick out of seeing their ideas actually transformed into a product, ensuring an adequate supply of employees. And third, Norway actually benefits from being small in that industry knows what SINTEF is, and can very easily get in touch with the appropriate people.

If there is a fourth important reason, I would say it is that a country like Norway simply doesn't have the national resources to turn research and development into a holy cow. Apply your research or starve. Perhaps one of the salutary results of Britain's tragic situation today will be precisely this.

Norman Sanders

Trondheim, Norway

EEC directives

Sir,—Eric Ashby (October 16) says that "all pollution except that from atomic weapons is a by-product of processes which benefit society".

Lord Ashby must have walked sometimes through a busy street and been assailed by a barrage of traffic noise from cars and heavy lorries (all necessary?) and breathed in their exhaust fumes. Perhaps he might have gone into a restaurant or cinema and filled his lungs with tobacco smoke. There is a vast amount of packaging of food in plastic and the like, much of it for the sake of advertising. Many people are engaged for eight hours or more per day with sheer monotonous routine work making products which have more to do with status than with benefit. Can Lord Ashby honestly believe, therefore, that much of what is manufactured in our society is beneficial?

Why is it a phony argument for the EEC to assert that a Scottish mill discharging into the Atlantic will unfairly compete with a mill on the Rhine which has to comply with stringent standards? Surely, if a uniform standard were not applied to all factories, then only those factories which could pollute the environment unabated would remain in existence while others under stringent control would go out of business. After all, Lord Ashby did mention that he was in argeement with the EEC's long term objective concerning the purity of the sea.

Lord Ashby did not like the idea of regular monitoring of the beaches for pollutants, and his main objection was that it would cost £100 million a year (a guess by the Department of Environment). He supports his objection by mentioning a report made by the Medical Research Council in 1959 which stated that, in spite of the smelliness and ugliness of many beaches, they were not a hazard to health. Even if the conclusion of the report were absolutely correct, has a report published 16 years ago any relevance to what is happening today and could happen in the future as regards the pollution of the sea?

The gist of Eric Ashby's article seems to me to be: 'We're not going to let a lot of foreigners tell us what to do'. The 'foreigners' seem, however, to be much more willing to tackle pollution than do the powers that be in Britain.

Paul C. French

Blommenholm, Norway

A hundred years ago

At last Friday's lecture by Dr. Carpenter, in connection with the St. Thomas Charterhouse School Teachers' Science Association, Dr Lyon Playfair presided. In proposing a vote of thanks to Dr. Carpenter, Dr. Playfair referred to the subject of compulsory education, which is gradually becoming universal in this country, but which, he said, would be pure tyrrany unless the education in our schools was increased and its quality raised Quantity is all very good, but unless there is quality along with it, there is not much gained "If it was to be said that children of thirteen or fourteen years of age were merely to receive the same education would be but tyranny. Therefore compulsory education involved higher education." Dr. Playfair expressed his gratification that the teachers composing the Association had banded themselves together in order to qualify themselves by attending such lectures as those of the Gilchrist fund and by other means, to undertake this higher education, which, we believe with Dr. Playfair, will be forced upon us even in elementary schools by the spread of compulsory education.

from *Nature*, **13**, 35, Nov. 11, 1875.

news and views

Humoral factors in myasthenia gravis

from Vanda Lennon

IN 1905 Buzzard speculated that the symptoms of myasthenia gravis (MG) might be caused by an "autotoxic agent which has a special influence on the protoplasmic constituent of voluntary muscle" (*Brain*, **28**, 438). Today two distinct protein entities have been identified as potential candidates for the role of the "autotoxin" mediating neuromuscular blockade in MG: antibody to acetylcholine receptor (*News and Views*, *Nature*, **256**, 10; 1975) and thymopoietin (formerly called thymin), a polypeptide derived from the thymus (Goldstein and Schlesinger, *Lancet*, **ii**, 256, 1975).

Purification of nicotinic acetylcholine receptor protein (AChR) from the electric organs of *Electrophorus electricus* and *Torpedo californica* allowed the establishment of an animal model of MG—experimental autoimmune myasthenia gravis (EAMG) (Patrick and Lindstrom, *Science*, **180**, 871; 1973; Lennon *et al.*, *J. exp. Med.*, **141**, 1365; 1975; Tarrab-Hazdoi *et al.*, *Nature*, **256**, 128; 1975). EAMG is readily induced in mammals by immunisation with AChR and resembles spontaneously occurring human MG by every criterion examined so far. Clinically, muscle weakness, fatigability and improvement by anti-cholinesterase drugs occur. Electrophysiologically, a decrementing response of muscle to motor nerve stimulation occurs at low rates of stimulation (2–10 s^{-1}) and rapid repetitive stimulation induces post-activation facilitation followed by exhaustion—phenomena which occur in MG The electrical defect is repaired by anti-cholinesterases and sensitivity to curare is pronounced (Seybold *et al*, *Ann. N.Y. Acad. Sci.*, in the press). The amplitude of miniature end-plate potentials (mepp's) is reduced, but the number of transmitter quanta released by a nerve impulse and the stores of acetylcholine quanta immediately available for release by nerve impulse are normal (Lambert *et al.*, *Ann. N.Y. Acad. Sci.*, in the press). Ultrastructurally, motor end-plates of rats with chronic EAMG are indistinguishable from those of patients with MG, the post-synaptic membrane being the primary target of the autoimmune reaction (Engel *et al.*, *J Neuropath, exp Neurol.*, in the press).

The critical link between EAMG and MG has been the finding of antibody to AChR in the serum in both conditions (Almon *et al.*, *Science*, **186**, 55; 1974; Aharonov *et al.*, *The Lancet*, **2**, 340; 1975; Lindstrom *et al.*, *Ann. N Y. Acad. Sci.*, in the press). Using human muscle AChR as antigen, Lindstrom and coworkers have found that anti-receptor antibody is specific for MG, being present in 87% of 71 MG sera tested (and also in newborn infants of a myasthenic woman) and in none of 156 subjects with other neurological, autoimmune and endocrine diseases. Interestingly, this antibody, like that in EAMG, is not directed at the acetylcholine binding site of the receptor. *In vitro* studies have shown, however, that anti-AChR antibodies from animals with EAMG diminish acetylcholine sensitivity of muscle fibres, reduce the mepp amplitudes and decrease the ability of AChR *in situ* to bind α-bungarotoxin (Green *et al.*, *Proc. R. Soc. Lond.*, **189**, 57; 1975).

Thymopoietin is a polypeptide which has recently been purified from bovine thymus (Goldstein, *Nature*, **247**, 11; 1974) and synthesised (Schlesinger *et al.*, *Cell*, **5**, 367; 1975). This polypeptide was named for its unequivocal capacity to induce bone marrow cells *in vitro* to express differentiation antigens characteristic of thymocytes (Basch and Goldstein, *Proc. natn. Acad. Sci. U S.A.*, **71**, 1474; 1974). Additionally thymopoietin is reported to have an effect *in vivo* on neuromuscular transmission (Goldstein, *Lancet*, **ii**, 119; 1968; Goldstein and Schlesinger, *ibid.*, **ii**, 256; 1975). When thymopoietin was given to mice intraperitoneally in nanogram amounts an apparent neuromuscular block (which did not increase with increasing doses) was found between 18 h and 5 d later. This contrasts with the effects of cobra venom toxins which block AChR *in vivo;* in rats these toxins cause weakness, which is dose related, and electromyographic decrements typical of MG beginning within 10-60 min and reaching a maximum by 3-13 h (Satyamurti *et al.*, *Science*, **187**, 1975; Lennon *et al*, *J. exp. Med.*, **141**, 1365; 1975). The characteristics of the decrement ascribed to thymopoietin have not been reported and are not evident in the short published record. Unfortunately, the defect was demonstrated at a stimulation rate of 50 s^{-1}, a rate at which artefacts are difficult to avoid; stimulation rates of 2-5 s^{-1} are optimal for demonstrating decrements in the diagnosis of MG (Slomic *et al.*, *Brain Res.*, **10**, No. 1; 1968; *New Developments in Electromyography and Clinical Neurophysiology*, Desmedt, J., ed., **1**, 241–304, Karger, Basel; 1973). Thus, the mechanism of action of this interesting polypeptide requires detailed electrophysiological analysis but its relevance to MG is far from clear.

What then is the role of the thymus in myasthenia gravis? Abnormalities of the thymus (hyperplasia and tumours) are often found in association with MG, and selected patients benefit from thymectomy. About 30% of patients with MG have antibodies, demonstrable serologically, which react with striations of skeletal muscle and with similar elements in the thymus, the "myoid" cells. Nicotinic AChR also has been identified in the mammalian thymus (Lindstrom *et al.*, *Ann N.Y. Acad Sci.*, in the press; Aharonov *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1456; 1975) Thymuses of patients with MG typically have germinal centres, which are the hallmark of antibody production and are not normally present in primary lymphoid organs. Thus it is not surprising that MG thymuses contain a large proportion of B lymphocytes (Abdou *et al.*, *New Engl. J.*

Med, **291**, 1271; 1974) and that in tisue culture MG thymocytes, in contrast to normal human thymocytes, synthesise considerable quantities of immunoglobulin (Smiley *et al.*, *Clin. exp. Immun.*, **4**, 387; 1969). It has yet to be ascertained whether this immunoglobulin is anti-AChR antibody, which would mean that in MG the thymus is a site of autoantibody production. It was the presence of germinal centres in MG thymuses that initially led Goldstein to propose that the primary lesion in MG was an autoimmune "thymitis" which led to the release of large amounts of thymopoietin. Goldstein and Whittingham (*Lancet*, **2**, 315; 1966) reported induction of histological signs of "autoimmune thymitis" in experimental animals by immunisation with saline extracts of muscle and thymus tissue in adjuvant. Those animals had no signs of weakness but did exhibit an electromyographic abnormality similar to that described in the subsequent thymopoietin studies. Small reductions in the amplitudes of mepp's were reported in rats with "thymitis" (Goldstein and Hofmann, *J. Neurol. Neurosurg. Psychiatr.*, **31**, 453; 1968). The reduction in amplitudes was much smaller than that observed in patients with MG or in rats with EAMG. Blind studies and attention to details such as differences in input resistance would seem essential in assessing the significance of the small differences reported in rats with "thymitis". On the basis of observations in EAMG, rats with so little reduction in mepp amplitudes would not be expected to show decrementing electromyograms. Considerable controversy has surrounded the model of "experimental autoimmune thymitis", some authors claiming confirmation of Goldstein's findings (Kalden *et al.*, *Clin. exp. Immunl.*, **5**, 319; 1969; Kawanami and Mori. *ibid.*, **12**, 447; 1972; Mrozek and Kaminska, in *Structure and Function of Normal and Diseased Muscle and Peripheral Nerve*, (edit. by Petrusewicz, I., and Jedrejowska, H., 145 Polish Medical Publishers, 1972), others failing to confirm the findings (Vetters *et al.*, *Lancet*, **2**, 28; 1969; Kaufman *et al.*, *J. Neurol. Neurosurg. Psychiatr.*, **32**, 281; 1969; Jones *et al.*, *J. Neurol. Neurosurg. Psychiatr.*, **34**, 399; 1971). It seems imperative that the effects of thymopoietin on neuromuscular transmission now be re-examined and documented in as convincing a manner as have been its T-lymphocyte differentiating properties. ☐

Teratocarcinoma cells can develop normally

from Brigid Hogan

TERATOMAS and teratocarcinomas are rare tumours which arise in the gonads, and contain a whole variety of differentiated tissues of ectodermal, mesodermal and endodermal origin (such as skin, nerve, muscle, cartilage, gut and lung), mixed together in a disorganised mass. Although tissue culture lines of teratocarcinomas have been available for many years, it is only recently that technical progress in studies of the biochemical properties and *in vitro* differentiation of these cells has enabled their potential as a model for early mammalian development to be assessed (for a recent review, see Martin (*Cell*, **5**, 229–243; 1975). The paper by V. E Papaioannou *et al* in this week's *Nature* (page 70) now elegantly confirms and extends the key observations that, placed in the environment of the early embryo, teratocarcinoma cells are in fact able to participate in normal morphogenesis and differentiation.

The study of teratocarcinomas was first put on a modern scientific basis in the late 1950s by Leroy Stevens in the Jackson laboratory, who noticed that the 129/Sv strain of mice had a high frequency of spontaneous testicular teratocarcinoma. Stevens and his colleagues showed that the tumours arose from germ cells in the testis and were retransplantable in either a solid or ascites form so long as nests of undifferentiated "embryonal carcinoma" cells were included in the inoculum; in the absence of these cells the transplanted tissue formed a slow-growing, benign, teratoma. The ascites form of the tumour consists of numerous small "embryoid bodies" floating in the ascites fluid; the simplest of these aggregates are made up of an outer layer of endoderm cells surrounding a core of embryonal carcinoma cells. This curious arrangement is in fact strikingly similar to the organisation of very early mouse embryos. At 4 days after fertilisation the normal blastocyst consists of a hollow trophoblastic vesicle enclosing a cluster of about 15 cells known as the inner cell mass (ICM); by 4.5 days the outer layer of the ICM differentiates into endoderm cells (which later form the extra-embryonic yolk sacs), while the internal cells go on to form ectoderm and mesoderm (the origin of the embryonic endoderm is at present unclear) Later work from a number of laboratories established that transplantable teratocarcinomas could also be obtained from many different strains of mice by injecting (male or female) embryos of up to 7 days development (but not older) into extrauterine sites such as the kidney capsule.

When embryoid bodies of either testicular or embryo-derived tumours are cultured *in vitro* they attach to the dish and cells proliferate out to form a multi-layered sheet within which a whole range of differentiated tissues gradually develop (Teresky *et al.*, *J. Cell Physiol.*, **84**, 319–332; 1974, and Gearhart and Mintz, *Cell*, **6**, 61–66; 1975, for recent examples of this process). Single embryonal cells can also be isolated from embryoid bodies and used to obtain a clonal population of homogeneous cells which remain undifferentiated over many generations, so long as they are prevented from forming dense aggregates. The majority of these cells retain a normal mouse karyotype and have several morphological, biochemical and cell surface properties (review by Artzt and Bennett, *Nature*, **256**, 545–547; 1975) in common with cells of the early mouse embryo.

Recently, G. R. Martin and M. Evans showed that if teratocarcinoma cells are allowed to form floating aggregates, the outer layer differentiates to form endoderm (*Proc. natn. Acad. Sci.* U.S.A., **72**, 1441–1445; 1975). So far, sorting out or differential proliferation of two cell populations has not been rigidly excluded. It seems more likely, however, that the cells on the outside of the aggregate receive a signal triggering them to differentiate as endoderm, a process which also happens during the normal development of the 4–4·5 day ICM. (Rossant, *J. Embryol. exp Morph.*, **33**, 991–1001; 1975). What kinds of signals are responsible for triggering such changes in cell committment is a crucial question in developmental biology, and if teratocarcinoma cells are to be of any use in solving the problem it is important to show that they are equivalent to embryonic cells in their ability to participate in normal morphogenesis and differentiation when placed in the environment of the early embryo. The first indication that such participation could occur was provided by R. L. Brinster (*J exp. Med.*, **140**, 1049–1056; 1974) who injected embryonal carcinoma cells from simple embryoid bodies from ascites fluid into 4 day mouse blastocysts of a different genotype and obtained one chimaeric animal. At a meeting held in May of this year at the Roche Institute, New Jersey, B. Mintz announced that she had successfully repeated this experiment, testing for chimaerism with several different genetic markers. In the experiments reported by Papaioannou *et al.* three lines of teratocarcinoma cells cultured *in vitro* have been used as a source of donor cells and out of 121 animals born from inoculated blastocysts 11 showed evidence for chimaerism as judged by glucose phosphate isomerase isozyme, melanin and agouti markers. A proportion of the chimaerae from one cell line developed tumours, and some tissues (blood for example) do not appear to be chimaeric, but overall these results are very good evidence that at least some cultured teratocarcinoma cells are able to participate in normal development. As Papaioannou *et al.* point out, one implication of their experiment is that it should now be possible to select mutants of the cultured cells and use them to study metabolic cooperation and cell interaction during development. ☐

Mismatched DNA and recombination

from a Correspondent

It is now generally accepted that structures containing regions of heteroduplex DNA, with one strand of the duplex contributed from each parent molecule, are essential intermediates in the biochemically complex process of genetic recombination. During heterozygous crosses, the inclusion of a genetic marker within such a hybrid segment of DNA will mean the formation of a mismatched base-pair, or some other localised distortion of the DNA structure. It has been clearly shown in several systems that localised aberrations in DNA structure are subject to enzymic repair, by processes presumed to be analogous to the excision of pyrimidine dimers from ultraviolet-irradiated DNA. This repair of heteroduplex mismatches is believed to be a fundamental step in the recombination process and to be primarily responsible for the unequal segregation of the components of an allele-pair in the process of gene conversion.

Ahmad, Holloman and Holliday, in this week's issue of Nature (page 54) have now demonstrated that a nuclease from the smut fungus, *Ustilago maydis*, which had previously been implicated in genetic recombination, has the properties expected of an enzyme involved in the repair of mismatching heteroduplices.

The enzyme, DNase I, purified from extracts of wild-type *U. maydis*, introduces single-strand breaks in linear, duplex and DNA molecules and reduces linear, single-stranded DNA to small oligonucleotides (Holloman, *J. biol. Chem.*, **248**, 8114; 1973). Ahmad *et al.* have now studied the activity of the nuclease towards linear duplex DNA molecules containing local distortions, using heteroduplex molecules made from the separated strands of the DNA of bacteriophage SPP1. The state of the DNA can be sensitively monitored through its efficiency in the formation of infectious centres on transfection of competent cells of *B. subtilis* Spatz and Trautner, who previously used this system to study the *in vivo* repair of heteroduplices (*Molec. gen. Genet.*, **109**, 84; 1970), have shown that a single-strand nick inactivates the DNA molecule in the transfection assay.

During a standard exposure of the DNA to the nuclease, the extent of inactivation of heteroduplices containing a single base-pair mismatch is about 6-fold greater than that of the homoduplices. Heteroduplices containing a small deletion on one strand are better substrates than those containing single base-pair mismatches. Thus the *Ustilago* enzyme is clearly able to recognise and preferentially attack those molecules containing distortions of the bi-helical structure. The nature of the biologically inactive products of the enzymic reaction was not investigated.

Well characterised nucleases from *Neurospora crassa* and *Aspergillus oryzae* have many similarities to the *Ustilago* enzyme, including the marked specificity for polynucleotides with disordered structures. The novel feature of the *Ustilago* enzyme is that it is strongly implicated in genetic recombination. Double mutants of *Ustilago*, isolated by screening for deficiencies in both extracellular and intracellular doexyribonucleases, show no detectable allelic recombination (Badman, *Genet. Res*, **20**, 213; 1972) and much reduced DNase I activity (Holloman and Holliday, *J. biol. Chem*, **248**, 8107–8113; 1973). The presumption that the deficiency in recombination and the loss of deoxyribonuclease activity are due to the same genetic lesions is supported by the finding that the two phenotypes are segregated together through meiosis.

The unusual specificity of these fungal nucleases makes them of more general interest as tools in molecular genetics. Their value is strikingly illustrated in a recent paper by Shenk, Rhodes, Rigby and Berg (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 989–993; 1975) who have used the *Aspergillus* nuclease, S1, to analyse mutations in the genome of the monkey virus, SV40.

Shenk *et al.* demonstrates that the S1 nuclease specifically hydrolyses linear heteroduplices of SV40 DNA at the position of a single-strand nick, a small denaturation loop or even, in some cases, a single base mismatch. This property should have remarkable ramifications in molecular genetics, since it potentiates accurate localisation of genetic lesions in systems devoid of genetic recombination and of lesions that cause no phenotypic change. It should also facilitate the isolation of small, discrete segments of a genome in the many cases where the segment can be bounded by two suitable mutations. Satisfactory S1 nuclease is already commercially available!

Evolution of tolerance to pollution

from Peter D. Moore

Although attention has recently been paid to the influence of pollutants on the structure and metabolism of whole ecosystems (for example Woodwell, *Science*, **618**, 429; 1970), the effects of pollution on species composition have been the subject of rather more research Detailed observational studies, such as those of Hawksworth and Rose (*Nature*, **227**, 145; 1970) on epiphytic lichens have even made it possible to estimate ambient pollution levels from the species composition of communities. Such studies demonstrate that different species differ in their tolerance to a given pollutant, some responding at lower thresholds than others But even within populations of a single species, the presence of a pollutant in the environment selects against those individuals which are most sensitive to it. This has been demonstrated most effectively in the evolution of heavy metal tolerance in grasses near abandoned mines (Antonovics, Bradshaw and Turner, *Adv. Ecol. Res.*, **7**, 1; 1971), but an increasing amount of evidence suggests that air pollutants are exerting a similar influence.

The classic work of Kettlewell (see *The Evolution of Melanism*, Clarendon Press, Oxford, 1973) on the development of melanism as a response to air pollution by particulate matter, has demonstrated that such selection occurs among insect populations The problem can be a complex one, however, as has been shown recently in the case of the two spot ladybird (*Adalia bipunctata*). Creed (*Nature*, **249**, 391; 1974) described the high incidence of melanism in this species in areas of high smoke pollution. He showed a distinct negative correlation between the frequency of melanic forms and the distance from a smoke-emitting 'Phurnacite' plant, which produces smokeless fuel from coal, in the valleys of South Wales. Subsequently, however, an alternative explanation of this correlation has been put forward by Muggleton, Lonsdale and Benham (*J. appl. Ecol.*, **12**, 451; 1975). They have reconsidered the distributional evidence and come to the conclusion that melanism in the two spot ladybird is more closely related to the duration of sunshine in any area, which, in turn, is likely to be affected by smoke levels. They supplement this with experimental evidence that the temperature of the melanic form rises more rapidly when exposed to a given level of radiation, thus providing it with a metabolic advantage in conditions of low insolation.

Recent work among plants has concentrated upon sulphur dioxide as an air pollutant. About 6 million tonnes of SO_2 are emitted into the atmosphere over Britain each year; thus it constitutes one of our major air pollutants. Prolonged exposure to low concentrations of SO_2 (below about 15 p.p.hm) does not seem to harm most plants, although depressed yield in ryegrass variety S23 (*Lolium perenne*) has been reported in experiments conducted at SO_2 levels below this (Bell and Clough, (*Nature*, **241**, 47; 1973). Since one of the most noticeable effects of SO_2 on plants is to cause increased stomatal opening (Unsworth, Bisco and Pinckney, *Nature*, **293**, 459; 1972), its deleterious influence may be indirect in allowing other toxic gaseous pollutants to enter the leaf tissue.

Sulphur dioxide pollution thus places a stress upon plant species and selects against those individuals in a population which are least tolerant. The effectiveness of this selective force has recently been demonstrated by the work of Taylor and Murdy (*Bot. Gaz.*, **136**, 212; 1975) in the vicinity of a coal burning power station in Georgia, USA. Seed was collected from a winter annual species, *Geranium carolinianum* at sites close to the station and at control sites some distance away, where SO_2 pollution was not a major stress. Nine weeks after germination, the plants were subjected to 12 h exposure to an atmosphere containing 80 p.p.hm SO_2. Three days later leaf damage was assessed for each individual on the basis of mean percentage leaf area showing necrosis. The method of assay should overcome the differential absorption of SO_2 which occurs in leaves of different ages (Craker and Starbuck, *Environ. Res.*, **6**, 91; 1973)

Frequency distributions of plants with varying degrees of leaf damage show a very considerable spread in all populations. It is to be expected that material derived from seed should exhibit wider variation than would the mature plants, since the latter would have been subjected to selection during establishment and maturation. Nevertheless, statistically significant differences were demonstrated between the mean leaf area damaged in populations near to and far from the source of SO_2 pollution. Differences were generally of the order of 10% less damage to the populations found close to the power station.

One can conclude, therefore, that there is a greater degree of tolerance to acute SO_2 pollution in the populations of *Geranium carolinianum* growing near a source of SO_2. It would be interesting to examine the relative tolerances of the populations to chronic exposure to lower SO_2 concentrations and to find out precisely what physiological adaptation is involved in the tolerance. This work indicates that very rapid selection (the power plant in question is just 31 years old) can occur amongst annual species, when subjected to a new environmental stress.

Transfer factor in viral infections

from Arie J. Zuckerman

THE transfer of cell-mediated hypersensitivity to tuberculin and streptococcal antigens was first achieved in man using blood leukocytes by Lawrence (*Proc. Soc. exp. Biol.*, **71**, 516; 1949). These observations in turn led to the finding that leukocyte extracts were as effective as viable cells for the transfer of delayed-type hypersensitivity. But these apparently innocent observations betray the controversy which surrounds transfer factor and which resulted in such sensational headlines as "Transfer Factor—science or magic?" (*New Scientist*, September 12, 1974) and "Transfer Factor—another scandal?" (*loc cit*, December 26, 1974). F. M. Burnet wrote recently:

> "Widespread recent interest in the transfer factor of H. S. Lawrence and its possible therapeutic applications has not yet led to an interpretation of the phenomenon that can bring it into a satisfactory relationship with standard immunological theory. The specificity of transfer factor (TF) has been demonstrated in the sense that only a small proportion of the lymphocytes in a recipient can respond to a given TF preparation. The active agent in TF is known to be of low molecular weight (4,000 to 5,000), but its chemical nature and the basis of its specificity are unknown. Neither is anything known about the cell receptor that must be capable of specific reaction with a particular TF. An hypothesis is developed which sees the biological function of TF as to recruit T lymphocytes to deal with situations such as delayed hypersensitivity or allograft rejection reaction for which there are inadequate numbers of immunologically reactive lymphocytes Although the specificity of TF does not appear to be related to immunological specificity, it calls for a genetically based generation of diversity roughly analogous to that required in the development of the immune system" (*J. Allergy clin. Immun*, **54**, 1; 1974)

The factual background to the controversy surrounding transfer factor is as follows. Transfer factor may best be described as a dialysable leukocyte extract that initiates and augments delayed-type hypersensitivity and cell-mediated immunity in normal persons, and Lawrence (*Adv. Immun.*, **11**, 195; 1969) considered transfer factor to be an antigen-specific polypeptide–polynucleotide molecule. Gottlieb *et al.* (*Lancet*, **2**, 822; 1973) found, however, that crude leukocyte dialysate from donors sensitive to *Candida* consisted, after concentration and elution from a column of Sephadex G 10, of three fractions, one of which was more active than the crude transfer factor. This fraction had a density of 1.47 g ml^{-1} in caesium sulphate and the properties of a short polypeptide chain consisting of perhaps 12 amino acids joined to 3 or 4 RNA bases in a ratio of 2: 1. The molecular weight of transfer factor is less than 10,000, probably 2,000—4,000. It is resistant to trypsin and mammalian DNase and RNase and the ultraviolet spectrum indicates the presence of polypeptide moieties. It is not related to any immunoglobulin class and it is non-immunogenic in man or animals. The present concept of transfer factor is therefore that of a soluble indicator of cellular immunity, for example an informational polynucleotide or a specific gene derepressor, which instructs previously uncommitted lymphocytes to become specifically sensitised and thereby transfers the state of delayed hypersensitivity. It has been suggested that cellular cooperation between T lymphocytes, B cells and macrophages may be associated with lymphokine factors and transfer factor for the induction of delayed hypersensitivity and antibody synthesis or in cytotoxic activity against cells with viral, tumour or histocompatibility antigens (Playfair, *Clin. exp. Immun.*, **8**, 839; 1971). In fact, Dumonde and Maini (*Clin. Allergy*, **1**, 123; 1971) suggested that, in the presence of a specific antigen, transfer factor induces non-sensitised lymphocytes to produce lymphokines.

Clinically, transfer factor activates uncommitted and non-sensitised lymphocytes of the recipient so that new clones of antigen-specific cells are produced. Besides conferring specific skin sensitivity, these cells may also produce interferon and other biologically active products which, as a result of non-specific stimulation, may contribute to the overall pattern of the cellular immune response in the recipient. It should be noted that transfer factor seems to be exceedingly potent; the amount of factor required to confer systemic skin reactivity on a normal recipient is the extract of only 0.1 ml of packed leukocytes. Reactivity to the other antigens to which the donor was sensitive, such as *Candida,* mumps, streptococcal and other antigens, is transferred simultaneously. But transfer of the capacity for antibody formation does not occur.

The clinical use of transfer factor is based on the view that depressed cell-mediated immunity is of pathogenic importance in various disease states and that, by reversing the deficiency, transfer factor can ameliorate the disease process. This has been tested in severe infections caused by mycobacteria, fungi and viruses, particularly in patients with defective lymphocyte responsiveness either *in vivo* or *in vitro.* On the same basis it has been possible to correct certain primary immunodeficiency states in some patients exhibiting selective depression of lymphocyte function.

Patients suffering from a number of infectious diseases, including mucocutaneous candidiasis, coccodioidomycosis, leprosy and a number of viral infections, have been treated with transfer factor. The patients responded to treatment with transfer factor obtained from donors in whom skin tests to the particular antigen were strongly positive. Successful outcome has been reported in a few patients with generalised vaccinia, herpes zoster, neonatal herpes virus infection, measles "giant-cell" pneumonia and subacute sclerosing panencephalitis.

Spitler and colleagues (*Clin. Immunobiol.,* edit by Bach, F. H., and Good, R. A., Academic Press, 1974, p. 153) treated a number of patients with verruca vulgaris, an example of a chronic viral infection, with transfer factor pooled from normal donors presumed to have immunity to this virus. Clinical improvement occurred in only one patient after several months of therapy so that the improvment may be quite unrelated to the use of transfer factor. A note of caution was added recently by Stevens and his associates (*Clin. exp. Immun.,* **21,** 520; 1975). Dialysed transfer factor, prepared from the leukocytes of a donor whose warts had undergone spontaneous regression, was used in the treatment of a child with Wiskott–Aldrich syndrome, a congenital immune deficiency. The child then had a spontaneous regression at multiple areas with warts. A similar relationship was observed in a pilot study in four otherwise healthy patients. But a randomised double-blind study of thirty patients failed to confirm a causal relationship between therapy with transfer factor and wart regressions.

Attention has also been directed recently to persistent infection with hepatitis B virus. Kohler and his colleagues (*Clin. Immun Immunopathol.,* **2,** 465; 1974) treated a healthy persistent carrier of hepatitis B surface antigen with lymphocytes from an individual who had recovered eight months previously from hepatitis B. Within 24 h there was an increase in the titre of antigen and of serum aspartate transaminase levels, an indication of liver damage. It was suggested that the recipient's liver cells were transiently injured by a cell-mediated immune response, which was insufficient to terminate the infection. Transfer factor prepared from the same donor had no effect on the patient's liver function tests. The biological activity of this material, however, was not evaluated and it may have been inactive. Another patient, an infant who acquired hepatitis from her mother at birth, was given transfer factor prepared from the mother's lymphocytes. On two occasions the preparation caused a prompt, moderate increase in both the antigen and transaminase levels. Subsequently, the liver function tests became normal and the titre of hepatitis B surface antigen was reduced by 95%. It is generally considered that cell-mediated immunity is important in terminating hepatitis B infection. Temporary falls in the levels of hepatitis B antigen have also been reported after the use of transfer factor in a few patients with antigen-positive chronic active hepatitis. Jain and his colleagues reported at the meeting of the British Society of Gastroenterology held in Oxford in September, 1975, the results of treating three patients with "specific" transfer factor prepared from subjects recently recovered from hepatitis B infection. One patient with chronic active hepatitis, and cirrhosis associated with antigenaemia showed no response to transfer factor prepared from a normal blood donor but "specific" transfer factor increased the number of T lymphocytes; a second similar patient also showed no response to "normal" transfer factor but "specific" transfer factor increased transiently the serum aspartate transaminase levels suggesting stimulation of cell-mediated immunity with a resulting hepatitic reaction. The third patient suffered from antigen-positive active chronic hepatitis complicated by primary liver cancer; he was receiving corticosteroid therapy and did not respond to transfer factor. It was concluded that although there was no alteration in the serum titres of hepatitis B surface antigen further trials wtih transfer factor seem justified.

Finally, Bullock *et al.* (*New Engl. J. Med.,* **287,** 1053; 1972) pointed out that there is a risk of transfer factor exciting generalised hypersensitivity reactions to disseminated microbial or other antigens and that, paradoxically,

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Earthquakes without precursors?

from Peter J. Smith

SOME shallow earthquakes are known to be preceded by significant decreases in P and S wave velocities (V_p and V_s) and the seismic velocity ratio V_p/V_s. One of the problems in using these effects as a basis for earthquake prediction, however, is that not all shallow shocks appear to give such warning. Reasons proposed so far for the lack of premonitory effects in certain cases range from failure to measure V_p and V_s at the appropriate times to possible variations of the V_p, V_s and V_p/V_s decreases when measured in different directions with respect to the trend of elongated cracks in the surrounding rock. But two new experiments carried out by Wang *et al.* (*Geophys. Res Lett.,* **2,** 309; 1975) now suggest that the apparent presence or absence of precursory phenomena may depend not simply on observational arrangements but on something much more fundamental.

In the first experiment, a block of dry 'Westerly' granite with an imposed normal load was subjected to an increasing shear load until fracturing occurred. Both V_p and V_s for waves travelling normal to the shear plane decreased before rupture, and dilation took place in the same direction V_p/V_s decreased from a 'normal' value of 1.7 to 1.4 in line with previous field and laboratory observations. In the second experiment, the granite block was first sliced in half and then subjected to normal and shear stresses to give stick-slip motion. But no V_p and V_s changes were observed either before or during sliding, and there was no dilation against the normal pressure.

Wang and his colleagues conclude from this that there must be appreciable variations in the faulting process leading to shallow earthquakes. The degree to which V_p and V_s decrease will then depend on the extent to which faulting is associated with dilation and fracturing. The implication is that prediction based on precursory seismic wave velocity variations will not be possible even for all shallow earthquakes.

Antibodies to polynucleotides in SLE

from Graham R. V. Hughes

JUST under two decades ago, four groups reported almost simultaneously the presence of antibodies against DNA in the serum of patients with systemic lupus erythematosus (SLE). While early studies used double stranded (ds) DNA, it was found that many, if not all, SLE sera also reacted against single stranded (ss) DNA. Indeed it is now apparent that a heterogeneous collection of anti-DNA antibodies exists, binding to a variety of sites on the polynucleotides (Arana and Seligmann, *J. clin. Invest.*, **46**, 1867; 1967; Cohen, *et al.*, *Clin. exp. Immun.*, **8**, 551; 1971). During the past six years, the measurement of DNA antibodies by the ammonium sulphate precipitation technique (Farr) has become a standard clinical tool in the diagnosis and management of SLE. While antibodies against ds DNA occur almost exclusively in SLE, anti-ss DNA antibodies are not specific for this disease, occurring in rheumatoid arthritis, drug-induced lupus and a variety of other conditions, as well as in some normal sera.

The agent most commonly used in DNA binding tests is ^{14}C-labelled bacterial or mammalian double stranded DNA, and therein lies the main obstacle to standardisation of results. It has proved difficult using these preparations to assess the contribution to 'native' DNA binding of single stranded regions in the molecule. A recent study by Samaha and Irwin (*J. clin. Invest.*, **56**, 446; 1975) used a variety of techniques in an attempt to further characterise the antigenic regions in mammalian DNA. Using MAK chromatography followed by enzyme removal of single stranded regions, they showed that purified mammalian DNA is a mixture of almost homogeneous ds DNA, and ds DNA with significant single stranded regions. The latter were found to be rich in thymine, complementing the earlier studies of Koffler and his colleagues (*J. exp. Med.*, **134**, 294; 1971) who showed that sera with antibody to ds DNA are inhibited by the synthetic single stranded polymer poly(dA), less so by poly(dT), and least of all by poly(dC).

These studies suggest that some antibodies in SLE are directed at the ends of the molecule containing short thymine-rich single stranded portions and possibly also at areas of bifurcation between ds DNA and AT-rich single stranded regions in ds DNA. Thus it seems that the variable binding activity reported with various SLE sera may have more complex explanations, not simply reflecting specificity towards ss or ds nucleic acid, but possibly also pointing to differential binding of antibody to specific regions of structurally heterogeneous DNA. Two possible ways of bypassing the problem of ss DNA binding might be the use of circular ds DNA (such as SV40 DNA) or the use of synthetic self replicating polymers such as poly (dAT). Neither of these antigens has yet received adequate clinical trial. In the meantime, the clinical significance of slightly raised DNA binding values in some non-SLE sera remains uncertain.

Apart from clinical considerations, delineation of the various polynucleotide antigen–antibody systems has more than academic interest. The renal disease in SLE results largely from immune complex deposition, with DNA–anti-DNA antibody complexes being of considerable pathological importance. Less clear, however, is the relative contribution and possible interaction of the various polynucleotide complexes. Discrepancies appear to exist, for example, between the presence of anti-ss DNA antibodies in rheumatoid arthritis and scleroderma, and the lack of glomerular immune complex deposition in the majority of patients with these diseases.

One of the earlier known manifestations of circulating immune complexes in SLE was the presence of cold precipitating globulins (Christian, Haltfield and Chase, *J. clin. Invest.*, **42**, 823; 1975) and in a recent study, Winfield, Koffler and Kunkel (*J. clin. Invest.*, **56**, 563; 1975) have returned to cryoprecipitation in an attempt to determine the specific concentration of anti-polynucleotide antibodies in cryoprecipitates relative to serum levels. Using haemagglutination techniques, they found that antibody against ds DNA and ss DNA were both enriched up to a 100-fold in some SLE cryoprecipitates, as compared with serum. Antibody to ribonucleoprotein (thought to be associated with milder or absent renal disease) was only occasionally concentrated in cryoprecipitates. In contrast, anti-ds-RNA, which was commonly detectable in high titre in SLE serum, was only minimally concentrated, and only in a minority of precipitates. These data are in broad agreement with earlier elution studies of SLE glomeruli, where again anti-ds-RNA was not detected.

The findings again indicate that different anti-polynucleotide antibodies have differing pathogenetic properties. While this has, in essence, been known for over a decade, the dissection of these antigen–antibody systems appears now to be gaining momentum, the clinical goal being more precise management of SLE.

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it may be necessary to use anti-inflammatory drugs during the early stages of treatment. In immune deficiency states there is a suggestion that uncontrolled lymphoid cell proliferation and autoimmune disease may be additional complications (Gelfand, E. W. *et al.*, *New Engl. J. Med.*, **289**, 1385; 1973, and Ballow, M. *et al.*, *J. Pediatr.*, **83**, 772; 1973). Lawrence (*Clin. Immunobiol., loc cit*) noted that "therapy with transfer factor (TF$_D$) as presently employed is empirical, since the precise mechanism via which this small molecule confers or uncovers a specific antigen–receptor site on the recipient's circulating lymphocytes remains to be clarified."

Immunotherapy with transfer factor for the persistent carrier of hepatitis B surface antigen or antigen carriers with chronic liver damage requires a cautious and carefully considered approach. The safety and effectiveness of such preparations should be evaluated as far as possible in animal model systems (*WHO Techn. Rep. Series*, No. 570, 1975). There is also, of course, a need to establish provisional standard reference preparations of transfer factor and studies on specificity are urgently required.

Carbon-13 NMR of proteins

from J. Feeney

IMPROVEMENTS in NMR instrumentation over the last few years have largely overcome the sensitivity problems which had previously retarded ^{13}C NMR studies of medium-sized molecules (molecular weight >1000). By using a combination of Fourier transform and proton noise decoupling techniques, high sensitivity natural abundance ^{13}C spectra can now be obtained for small peptides such as oxytocin, angiotensin and LHRH in which each carbon features as a single sharp resonance signal. For larger peptides there is considerable overlap of the ^{13}C spectral lines and when a small protein such as lysozyme is examined relatively few single ^{13}C resonances can be resolved.

Professor A. Allerhand and his coworkers (Oldfield *et al.*, *J. biol. Chem.*, **250**, 6381; 1975) have now reported methods which allow them to detect most of the aromatic quaternary carbons in small proteins. By using a proton noise decoupling field slightly off-resonance for optimum proton decoupling only the quaternary carbons are efficiently decoupled and the sharp signals from these carbons can then be separated from the very broad bands from proton-bearing carbons using convolution difference methods (Campbell, *et al.*, *J. Magn. Res.*, **11**, 172; 1973). The resulting spectra feature only the quaternary carbon signals. Allerhand and coworkers have applied these techniques to several native proteins (lysozymes, cytochromes *c* and myoglobins) and using concentrated aqueous solution (10–20 mM) in the 20 mm diameter sample tubes at 15.18 MHz they are able to obtain high quality spectra in ~5 h of accumulation time.

Many assignments of the signals could be made by consideration of chemical shifts, relaxation behaviour in H_2O and D_2O, selective proton decoupling experiments, *p*H dependence, deuterium isotope effects on chemical shifts and line broadening induced by bound lanthanide ions. For hen egg white lysozyme the γ-carbons of the 6 Trp residues have chemical shifts over a range of ~5 p.p.m.; when the protein is denatured with guanidine the signals coalesce into a single absorption band. In the ^{13}C spectrum of horse heart cyanoferricytochrome *c* all 18 non-protonated aromatic carbon signals are resolved. When the diamagnetic forms of haem proteins such as horse heart ferrocytochrome *c* are examined, the haem carbons are also detected in the spectra but these disappear in the spectra of the paramagnetic form. In mixtures of ferro- and ferricytochromes *c* there is fast electron transfer which produces exchange effects in the averaged spectrum such that a 1 : 1 correspondence between non-protonated aromatic carbon signals from the two forms can be established.

Clearly one can use the assigned ^{13}C signals in the various proteins to monitor protein unfolding, ionisation states of specific residues and interactions between the protein and other molecules. It should be borne in mind, however, that studies of ^{13}C at natural abundance (1.11%) have the disadvantage that they require large amounts (50 mM per sample) of soluble protein.

An alternative approach is to enrich the protein selectively with ^{13}C-labelled amino acids. In this way, the concentration and solubility requirements (<1 nm solutions can be examined) are reduced and a much wider range of proteins can be studied. Several workers have demonstrated that ^{13}C-labelled amino acids can be incorporated into bacterial proteins from auxotrophic organisms. An elegant example is found in the work of Hunkapiller and coworkers (*Biochemistry*, **12**, 4732; 1973) who isolated α-lytic protease from *Myxobacter* 495 grown on a medium containing all the naturally occurring amino acids including 2-^{13}C-labelled histidine. There is only a single histidine residue in α-lytic protease (His-57) and thus there is no assignment problem for the intense signal obtained in the protein ^{13}C spectrum. The C-2 carbon is coupled to the directly bonded proton and the measured $^1J_{CH}$ coupling constants are characteristic of the ionisation states of the imidazole ring ($^1J_{CH}$=219 Hz when the ring is protonated and $^1J_{CH}$=206 Hz for the unprotonated ring). Thus they were able to show from such measurements that the *p*K of His-57 is very low in the enzyme (*p*K <4), a fact which has important implications in understanding the mechanism of action of serine proteases.

Usually the incorporated labelled amino acid occurs at several different sites in the protein and one is faced with the difficult task of assigning the ^{13}C signals. A general solution to the problem of observing and assigning ^{13}C signals from proteins will be available when semisynthetic proteins can be made by incorporating synthetically prepared fragments containing several different labelled amino acids in a region of interest in the protein structure.

Tumour viruses discussed at Copenhagen

from Ian Macpherson

The Seventh International Symposium on Comparative Research on Leukaemia and Related Diseases was held in Copenhagen on October 13–18.

ONE of the most important issues raised at the meeting concerned the true nature of the C-type viruses apparently isolated from the leukocytes of a human case of acute myeloid leukaemia by Gallagher and Gallo (*Science*, **187**, 350; 1975) also from the bone marrow cells of a child with lymphosarcoma by Nooter *et al.* (*Nature*, **256**, 595; 1975) and from human embryo lung cells passaged in culture by Panem *et al.* (*Science*, **189**, 297; 1975). These viruses were recognised as typical C-type particles like those known to cause animal leukoses and were found to have antigenic and reverse transcriptase affinities with simian sarcoma virus (SiSV). These relationships were not considered to be surprising or even unexpected but what raised a few eyebrows at the meeting were the observations made in several laboratories using competition radio-immunoassay analysis and molecular hybridisation, that both Gallagher and Gallo's and Panem's isolates each contain two distinct viruses with uncomfortably close affinities to SiSV and the baboon endogenous virus At present it is not possible to say who got what from where and when. The forthcoming conference of the Virus Cancer Program at Hershey may help clarify these uncertainties.

Regardless of how these questions are answered there is evidence from other sources suggesting that man commonly encounters viruses antigenically related to the murine, feline and/or simian agents. H. W. Snyder (Sloan-Kettering, New York) found that most, if not all, adult human sera contain antibodies that combine with polypeptides from these C-type RNA tumour viruses. Placental cord sera are negative and sera from leukaemic patients reacted like normal sera. These results suggest that a virus or viruses with antigenic specificities common to the animal C-type viruses used as probes in this study are widespread in the human community. R. S. Metzgar (Duke University Medical School) reported antigenic relationship between RNA tumour viruses and surface antigens of leukaemic cells in cytotoxicity tests. He has shown that human leukaemic myeloblasts have antigens related to the p30 and gp70 proteins of Friend mouse leukaemia virus. R. A. Lerner (Scripps, La Jolla) has found that gp70 has a remarkable pleomorphism in the mouse. By immunofluorescence he has shown that this component of mouse leukaemia virus surface coat is also present in mouse lymphoid and epithelial cells and in the secretions of the epididymis. The system offers an excellent model for the study of host control of this gene's expression.

An interesting observation that may provide at least a partial explanation why C-type viruses are not as readily detectable in man compared with other animals also came from studies carried out at Scripp's: F. C. Jensen provided evidence that human complement, without the intervention of antibodies, was capable of lysing a wide range of C-type viruses. This may provide man with an important line of defence against infection with viruses of this type.

P. H. Duesberg (University of

California, Berkeley) described further progress his group has made on the mapping of avian tumour viruses. The 30–40S RNA, which they have shown to have a 3′ poly(A) end, was fragmented with alkali. Fragments with the poly(A) tag were separated according to size and the location of a particular oligonucleotide relative to the poly(A) end was deduced from the smallest piece that could be found. Different virus strains were found to share certain oligonucleotides and these were in approximately the same linear location. Comparisons between transformation defective, and virus envelope defective mutant viruses with their respective wild-type progenitors have shown that the transformation gene(s) (the *onc*) is located near the poly(A) end and that the information for the virus envelope (*env*) is nearby. The continued application of this type of analysis in the hands of such able interrogators should eventually provide a complete picture of the genetic map of these viruses.

Another intriguing probe into the avian sarcoma viruses and their dealings with the cell was described by D Stehelin (University of California Medical Center, San Francisco). By making a radioactive DNA complementary to the whole RNA genome of a transforming virus and "absorbing" it with the RNA of a mutant virus without the transforming functions they were able to make a cDNA *onc* gene probe. Leukosis viruses and transformation defective mutants lacked these sequences. Uninfected cells of a variety of avian species including duck (which so far has not been found to contain an endogenous virus) contain the nucleotide sequences of the probe in their DNA but do not transcribe the gene into RNA. However, their finding that methylcholanthrene induced tumour cells from the quail contained homologous RNA to their probe is of considerable interest and hints at a link between chemical induction of tumours and the activation of "viral" genes.

The small DNA tumour viruses were brought back into the arena by H. zur Hausen (Universität Erlangen-Nürnberg). He has shown that human genital and laryngeal warts which sometimes undergo malignant transition were not found to have any measurable homology with a complementary RNA prepared from plantar wart DNA. This suggests that there are a variety of papilloma viruses in man, some of which may be responsible for malignant tumours This field may be due for a revival especially since there has been a recent report by Eisinger *et al.* (*Nature*, **256**, 432; 1975) that human wart virus can be grown in tissue culture. □

Cell motility moves ahead

from E. W. Taylor

The most recent conference in the Cold Spring Harbor series on Cell Proliferation, entitled Cell Motility, was held on September 9–14.

A KNOWLEDGE of how cells move and how their movements are controlled is necessary for an understanding of a variety of biological processes including embryological development, the wiring of the nervous system, and the invasive transformation of the cancer cell. During the past few years the number of papers devoted to some aspect of cell motility has increased enormously. The conference was a largely successful effort to establish communication among the practitioners in this diverse field The topics included muscle contraction, non-muscle actomyosin and streaming, structure and function of animal and bacterial flagella, chemistry and assembly of microtubules and mechanisms of mitosis.

Three main classes of motile systems can be distinguished on the bases of protein composition and energy transduction mechanism, the bacterial flagellum.. the eukaryote flagellum, the actomyosin system of muscles and cytoplasmic streaming.

The mechanism of bacterial flagella movement has long been a puzzle since the flagellum consists of a single protein with no known enzymatic activity. It has been shown that the flagellum does not propagate helical waves but rotates, driven at the base by a structure in the membrane. The sense of rotation can be reversed, producing swimming in the opposite direction if a single flagellum is present or preventing swimming by untwisting the flagella bundle. A physical analysis of the motion was presented by H C. Berg (University of Colorado) and the experiments establishing the rotation mechanism and the properties of motility mutants were discussed by M. Simon (University of California, San Diego) and J. Adler (University of Wisconsin). D. E. Koshland (University of California, Berkeley) described elegant mixing experiments which established the point that the chemotactic response was elicited by a change in concentration with time rather than a spatial concentration gradient across the bacterium. These studies lead to a simple model of chemotaxis which should be widely applicable to the movements of animal cells. The frequency of reversal of the motor is reduced by an increase in concentration of attractant. In simplest terms, if a bacterium is moving up a concentration gradient, motion in this direction will persist longer. If it moves down the gradient it will stop in a shorter period of time and try a new direction. As a result the step lengths in a random walk are biased in the direction of the concentration gradient.

The sliding filament–swinging cross bridge mechanism of muscle contraction has been firmly established and a reasonable correlation has been made with corresponding biochemical events in ATP hydrolysis by actomyosin. In recent years actomyosin has been isolated from a variety of tissues and organisms (brain, platelets and fibroblasts of vertebrates, slime mould, amoebae and algae). It is very likely that actomyosin is responsible for cytoplasmic flow and actomyosin-containing extracts of amoebae, studied by D. L. Taylor (Harvard University), mimic the flow properties of the intact cell. Several laboratories have studied the properties of cellular actin and myosin and a pattern is beginning to emerge. While the actin and myosin are similar to but distinguishable from their muscle counterparts, the regulation of ATPase activity and aggregation into gels depends on factors which are different or absent from the muscle system. Preliminary characterisation of protein factors which regulate actin polymerisation, aggregation of actin into bundles and formation of gels has been achieved in slime moulds by S. Hatano (Nagoya University), M. R. Adelman (Duke University Medical Center), J. A. Spudich (University of California, San Francisco), in amoeba by T. Pollard (Harvard Medical School) and in macrophages by T. P. Stossel (Children's Hospital Medical Center, Boston). Regulation of ATPase activity by Ca requires a protein factor which is distinctly different from troponin or a myosin light chain. Recent studies on this factor in amoeba and macrophages were reported by Pollard and Stossel respectively The field has passed out of the stage of counting up the number of systems which contain actomyosin to go on to the determination of the special properties of actomyosin systems which are peculiar to cytoplasmic flow.

Bundles of actin filaments are common cellular components but under some conditions a lattice is formed with α-actinin (a muscle protein) anchoring the crossover points of the lattice. Fluorescent antibody staining developed by E. Lazarides (Cold Spring Harbor) has enabled these complex networks to be made visible. At present we are suffering from a surfeit of actin filament bundles which seem to be present even in the mitotic spindle. These studies have reopened the question of whether actomyosin participates in chromosome movements (the question was not settled). The possibilities of artefacts in preparing cells for antibody or heavy meromyosin (HMM) labelling was keenly discussed and although there is growing evidence for the presence of actin, myosin has not been demonstrated. The studies of L. G. Tilney (University of Pennsylvania) have shown that actin is present on its own in the acrosome bodies of sperm from a variety of species and is driven into the egg by polymerisation or uncoiling. Thus the presence of actin in a cell organelle need not imply the presence of myosin as well.

Some three years ago, R. C. Weisenberg (Temple University, Philadelphia) developed a method of polymerisation of tubulin into microtubules. This work stimulated an intense study of the mechanism of microtubule polymerisation and the search for microtubule organising centres. Condensation of tubulin into outer doublets of flagella and asters of the mitotic apparatus has already been described and evidence was presented (by two different laboratories) for the chromosome kinetochore as an organiser. Microtubule polymerisation has turned out to be a complex process, more like virus assembly than the type of spontaneus polymerisation that occurs with fibrous proteins (actin for example). The formation of a ring structure by a protein factor or possibly polyvalent ions, the use of the ring as a nucleating centre, the participation of high molecular weight factors, and enzymes (transphosphorylase and possibly a protein kinase), the requirement for nucleotide triphosphate hydrolysis, as well as three different polymerisation models (H. P. Erickson–M. Kirschner (Duke University), G. G. Borisy (University of Wisconsin), M. Jacobs *et al.* (King's College, London)) were discussed. While it is clear that rapid progress is being made on this problem, more rigorous purification of the system is necessary before the discrepancies between different groups can be resolved. ☐

Transient observations of X-ray sources

from John Gribbin

At the Royal Astronomical Society meeting on October 10 the nature of the transient X-ray source Ariel 0620-00 was discussed.

THE discovery of the source early in August was described by M. Turner (University of Leicester). There was a rapid rise in intensity, with one slight hiccup, which made A0620-00 three times brighter than Sco X-1 by the middle of the month (see *Nature*, **257**, 656, 657; 1975). The hiccup, or 'precursor peak' seems to have marked a change in the energy production mechanism, with a fall in temperature preceeding the most rapid period of increasing brightness, and with the bulk of the increase occurring at low energy (below 10 keV) making this a very soft source. No lines have been found in the spectrum, although searches have been made especially at frequencies appropriate for ionised silicon and sulphur, and there is no evidence of polarisation at what Turner described as "the lowest upper limit yet achieved in X-ray astronomy" and there is no evidence of periodic variations anywhere in the range 0.2 ms to 2 d.

But there are some positive interpretations of the X-ray evidence, not least a SAS 3 measurement of the hydrogen column density to the source which implies a distance of 3 kpc if all absorption is interstellar, suggesting an absolute luminosity of 10^{39} erg s^{-1}.

A0620-00 has also been detected at radiofrequencies and identified with an optical star (see *Nature*, **257**, 659; 1975). R. Davis (Jodrell Bank) described radio observations both at Manchester and elsewhere which help to provide a very accurate position in right ascension, although the declination is less well determined because of the inconvenient position of the object for such measurements. The source is variable, and declined from peak intensity after August 18 with an *e*-folding time of 5 d, considerably faster than the equivalent X-ray decline (1/*e* fall off over 30 d). Assuming the outburst began on August 3, the radio data can be fitted by a T^{-3} power low.

The optical observations were described by M. Penston (Royal Greenwich Observatory), who preferred to call the event Nova Monoceros 1975 but agreed that he was indeed talking about the same thing. There seems no doubt at all about the identification of the optical and X-ray novae, and comparison with Palomar Sky Survey plates shows that during the outburst the source increased from a magnitude of 20.5 to 11.5, a brightening which is on the high side for dwarf novae but not unprecedented. The Palomar plates also show that the star was very red before the outburst, suggesting that it may be a red dwarf only some 500 pc away or a giant at the implausible distance of 15 kpc which, in the direction of Monoceros, would place it not only well out of the Galaxy but far above the galactic plane. At optical frequencies, the fade of the source has been little slower than in the X-ray band but faster than at radio frequencies, indicated by measurements up to a few days before the RAS meeting.

For the modellers, one of the most significant contributions comes from a search of Harvard plates which show a previous outburst in 1971, reaching 12 mag. This strongly implies the presence of circumstellar material leftover from that event, suggesting that the SAS 3 distance must be an upper limit and pinning the object down clearly as a red dwarf probably closer than 1 kpc.

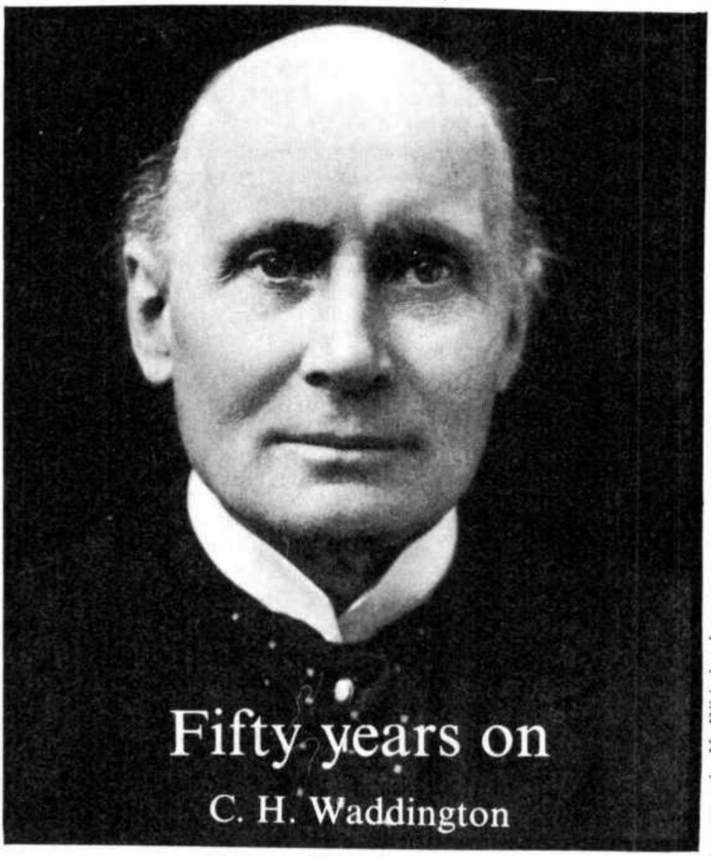
A. Fabian (Institute of Astronomy, Cambridge) took up this theme in presenting a model of A0620-00. Pointing out that this is the first optical identification of a transient X-ray source, he made a leap which perplexed some optical astronomers—but not the X-ray specialists—by assuming the now basic model of a binary source with mass transfer from the visible star onto a compact companion, probably a neutron star. The probability of seeing an eclipse on such a model is only 12%, so it is not surprising that none is seen in this source, and the increase in brightness of the dwarf is caused by the heating effect of X-radiation from the companion on one face of the larger star. The fact that novae seem to recur in this system encouraged Fabian to make a comparison with the recurrent nova WZ Sge, itself a similar binary system, and although this detailed model is unlikely to fit all the transient X-ray sources it is interesting that a very similar model has already been proposed for the archetypal X-ray source Sco X-1, again drawing a comparison with WZ Sge.

This raises the question of just how different the transient X-ray sources are from the rest of the family. All of the known X-ray sources that are not supernova remnants show very erratic behaviour with flares and quieter periods, and if Sco X-1 can be explained, after more than 10 years observation, by a model similar to that appropriate for a newly discovered source which flared into prominence for only a few weeks it seems that the differences may well be of degree rather than of kind. The observations themselves are far from continuous, and the label hung upon any particular source can depend to a large extent on the state it happened to be in when it was first noticed. Perhaps the main lesson to be learned from the story of A0620-00 so far is that all X-ray binaries are variable, but some vary more than others, and that rather than talking about "observations of transient X-ray sources" we would do better to talk about "transient observations of X-ray sources". ☐

Michael Shillaker

Fifty years on

C. H. Waddington



A. N. Whitehead

WHEN asked to write on the books that scientists were reading when I was young, I thought at first that it would be impossible to make anything of interest out of what I personally had been reading. There are, however, some points which may have some wider relevance. We will be dealing with the years 1925–30, when I was 20 to 25. That was just before many things happened, which we now accept as in the very nature of things.

When I ask myself "what was that time *before*?", I have to think of a very wide range of different topics. At that time it seemed absolutely natural that a young man of my age, interested as all such people are, in the nature of the world in which he was beginning to find his feet, should expect to find out about a great many different aspects of it. Not only his own science, but several sciences connected with it, even if only loosely. My 'official' interest was in what fossils can tell us about the processes of evolution. There was not very much general reading in that connection. That was *before* the development of mathematical neo-Darwinism, although J. B. S. Haldane was beginning to do some elementary sums about the selection of single genes, which he, rather sensibly, treated rather as undergraduate *jeux d'esprits* than as the profound contributions to fundamental biology which people later—and still—pretended them to be.

It was also absolutely natural to have interests in philosophy, poetry, even painting, and to allow them to show. This was well *before* there was considered to be any firm dividing line between the natural or the moral sciences, or even between those and the arts. It is in these areas that some of the *befores* are most difficult to clarify. Eliot's *The Waste Land*, and some of Ezra Pound had already been published, but it was *before* their influence had changed the whole direction of English poetic taste, at least as far as I was concerned; at that time, I was still reading English poets of the generation of Wilfred Owen, Edward Thomas, Gordon Bottomley, Walter de la Mare, the Sitwells and the American experimentalists and imagists, such as e e cummings, Laura Riding, Hart Crane, John Crowe Ransom and H.D.

But most important of all, certainly for my future development, were books by two people who were closely associated and who brought literary criticism and philosophy very near together. The official philosophers in Cambridge, such people as Broad and Moore, had already some of that remoteness which later became almost the hallmark of the profession. But this was *before* the influx of that remarkable succession of Viennese intellectuals which have dominated British philosophy since the early thirties—Wittgenstein, the first, best, and only one to recognise for himself that he had been talking nonsense and to spend the rest of his life unsuccessfully trying to get it right; followed by Schlick and Karnap, with their first major British follower, Ayer; then Popper, and Lakatos and now Feyerabend, whose book, *Against Method* (NLB London, 1975), should be the expiring gasp of the lineage of thinkers who set out to describe the definitive method of science in terms of logical algebra.

The two people I refer to, just *before* these, were I. A. Richards, with his *Principles of Literary Criticism* followed a few years later by *Practical Criticism*, and C. K. Ogden, who collaborated with Richards in *The Meaning of Meaning*, which was much more philosophy than criticism. Ogden also introduced us to, at least some, of the problems of linguistics, first from his own viewpoint, by the attempt to develop a "minimum effective language" (Basic English), but later using the fashionable Viennese ideas, as the first translator of Wittgenstein's *Tractatus*. I doubt if, today, you would find anywhere the intimate interplay between poetry, philosophy and the foundations of science which Ogden and Richards displayed.

The other main influence on me as a young man just growing up as a scientist, was that of the philosopher, A. N. Whitehead, the writings of whom some younger biologists seem to associate with molecular biology, or the even more recent influences of Levi-

Straus and Chomsky. He was the senior author, with Bertrand Russell, of the great tome on *Principia Mathematica* which laid the foundations of philosophy in the mode of logical algebra. His later treatment seems to me to border on the fantastic. It was Russell who persisted in the directions adumbrated in the *Principia*, and that work seems nowadays to be considered virtually his alone. In fact, Whitehead scarcely gets mentioned in most modern outlines of philosophy except as Russell's co-author in that work.

Admittedly, Whitehead did not make it easy for readers of later generations who were in a hurry to catch up. For instance, R. F. Atkinson (Twentieth *Century Mind*, **2**, Oxford), dealing with British Philosophy, from 1918–45, finds little more to say than: "His major metaphysical works are *Science and the Modern World* (1925), *Adventures of Ideas* (1933), *Process and Reality* (1929)—the latter very difficult work being the most complete statement of his views—...and a vast expenditure of time is needed to come to terms with his system. His opponents therefore ignore him instead of criticising him in detail". This is as though some one felt he was duty bound to assess the literary worth of James Joyce, and decided, after plunging into *Finnegan's Wake* for half an hour, that Joyce was not worth bothering about.

One should approach *Finnegan's Wake* through *The Artist as a Young Man* and *Ulysses;* one should approach *Process and Reality* through *The Principles of Natural Knowledge, Symbolism, its Meaning and Effect* and others of Whitehead's earlier writings, including *Science and the Modern World* (recognising that, however, as being something of a popularisation). Although I took this course in both cases, I confess I never quite made the grade to a confident mastery of either of the two *magna opera*—both a bit too big for their boots. I will go along with Atkinson that he is a difficult author, but that is more because his ideas are unfashionable and profound than on account of his readiness to redefine words to suit his own purpose.

Whitehead totally inoculated me against the present epidemic intellectual disease, which causes people to argue that the reality of anything is proportional to the precision with which it can be defined in molecular or atomic terms. Whitehead's main thoughts were *before* quantum mechanics; his refusal to grant prior validity to matter or atoms was not so much because no one knows what they are, but rather because all our knowledge has its roots in experience. Science is not based on physical or material realities, it is based on experiments. An experience is something more immediate than the distinction we are immediately tempted to make within it, into the objective and the subjective; it contains both of them.

Whitehead's major thesis was the rejection of what he called "The Bifurcation of Nature", into Mind and Matter, set against one another as two totally separable and incomparable essences. Of course it is often convenient to work with a rough and ready distinction for particular purposes. But he was—and I am—totally opposed to an unbridgeable gap between them—the "Cartesian Dualism", the "Naturalistic Fallacy", and all the rest of the rigmarole which is bringing science into such disrepute with the open-minded younger generation of today. If I had started reading philosophy a few years later, Whitehead would already have been pushed out of fashion by Wittgenstein and Russell, and I would probably have failed to grasp this point. ☐

Professor Waddington died shortly after completing this essay. An obituary will appear in a later issue.

Festschrift

Arthur Koestler. Photo: Michael Brett

Astride the Two Cultures: Arthur Koestler at 70. Edited by Harold Harris. Pp. 219. (Hutchinson: London, 1975.) £5.50.

I do not see how it can help but look singularly graceless to take up a critical stance towards *Astride The Two Cultures*, a *Festschrift* for Arthur Koestler on his seventieth birthday. Graceless or not, I have to disclose immediate disappointment at not finding a contribution originating in what I nowadays call the hard-core sciences, those grouped round mathematics and physics.

When C. P. Snow coined the phrase "The Two Cultures", it was clearly the hard-core sciences which he saw at the one pole; and Mr Koestler in his own writings handles some of the concepts of theoretical physics. The contribution coming nearest is by W. H. Thorpe, the distinguished animal ethologist: he deals with Mr Koestler's views on reductionism and with his ideas about the hierarchical nature of 'living organisation', making use in his frame of reference of the philosophies of Karl Popper and Jacques Monod—good hard-core thinking. Naturally Professor Thorpe is not the only writer to notice the parallel between Mr Koestler's ideas about hierarchy and General Systems Theory. (Briefly: Mr Koestler remarks that an element of a living organisation seen from one point of view appears as a non-autonomous part of the whole, but from another point of view as an autonomous individual.) As a literary metaphor it's nice, and falls in with non-deterministic principles, but it suffers from the limitations of literary metaphor. Theoretical physics may be called a mathematical metaphor: the difference is that mathematical metaphor enables you to penetrate to deeper levels of physical understanding, whereas literary metaphor only enhances your view on one level. As with Bohr's statement of Complementarity—it's nice, the more you look around you the more examples you see: but how much further forward does it get you? Thus, but more so, with Mr Koestler's "Janus-faced holons".

Under the current English dispensation you're expected to address yourself, in contributing to a Festschrift, to the work of the person being celebrated: under the original German dispensation you get away with printing large slabs of your own work—three of the present social scientist contributors operate on original German lines. One improves the shining hour with polemic against one of his academic colleagues—yes, Academe is located in the provinces. Another provides a large slab about personality traits: I'm sure diagnosing and assessing personality traits finds honest work for psychologists; it's bad luck that the upshot happens to be lifeless. A third studies animal behaviour: often I strongly deplore this kind of study, because it carries a *sub rosa* message of excuse for us human beings doing what the animals do. In this contribution the animal is a species of monkey whose males impress each other by exhibiting their phalli: if there's the *sub rosa* message here, it would seem to me harmless, in fact favourable to the spread of general merriment.

Atop the other pole—the idiom of the title is catching—sits Goronwy Rees with an excellent appraisal of *Darkness At Noon*. I've never been able to understand why first becoming a Communist and then becoming an anti-Communist should attract a special kind of moral credit such as is not accorded to someone who has had the sense to do neither. Performing two intellectual U-turns may purify a man's spiritual nature, but it doesn't establish him in my opinion as a reliable thinker. What it may do, it seems, is provide him with material for art. Generally speaking the question "Does the end justify the means?" is most suited to a College Debating Society, the answer being given by a count of Ayes and Noes. Mr Koestler, as Mr Rees demonstrates, has transformed an examination of the question into a work of art. I should have thought Mr Koestler's argument was skewed intellectually by the pressure of abnormally strong feelings of guilt—you really can't get to the truth when there's so much guilt about the place—yet as a work of art the book is clearly a classic. But I do feel bound to look back again to the opposite pole, where I find it hard to go along with the claims made for Mr Koestler there. He is extremely clever and he knows what some of the concepts of the hard-core sciences are about: I take leave to question very seriously the uses to which he puts them.

Of recent years Mr Koestler has become increasingly preoccupied with the so-called 'para-' subjects—telepathy, telekinesis, parapsychology and so on. In writing for *Nature* about Aldous Huxley, who also came to have this preoccupation, I lumped them all together under the title 'parascience', where 'para-' according to the Oxford English Dictionary meant 'beyond, wrong, irregular' and according to me could be replaced by 'non-'—'non-' being at only one stage before 'anti-'. In *The Roots of Coincidence* Mr Koestler begins by trying to establish parascience on the same footing as science: by saying that theoretical physics breaks previously sacrosanct 'laws of nature' in for instance the concepts of negative mass and time reversal, while parapsychological research has become more rigorous, statistical, computerised. A specious argument: on the one hand negative mass and time reversal are straightforwardly within the logic of the established mathematical metaphor; whereas everybody knows you can be rigorous, statistical, computerised from premisses that are entirely fallacious. Mr Koestler then goes on to postulate a world of psychic reality alternative to that of physical reality (and interwoven with it in the matrix of 'oceanic reality'; complementarity is invoked, of course), where the seemingly acausal events of the paraworld—amazing coincidences, peculiar premonitions, levitating tables and crumpling spoons—may be as accounted-for as the casual events of the physical. The world of 'oceanic reality' you reach by way of mystical or mescalin experiences.

One can readily take a magnanimous line towards Mr Koestler as a man. For instance when he flirts in *The Midwife Toad* with Lamarckism, one can see his sense of justice compelling him to establish the claims of dissident off-beat minds in the dialectical process of arriving at scientific truth. And when he attacks determinism one can only respect that element of the human spirit which insists on having some say in its own fate, and which rejects the idea of absolute predetermination and inevitability. But one's attitude to him as a thinker has to be cautious, clever as paint though he may be. One has to watch out for preoccupation with the paranormal turning a man into a parathinker. **William Cooper**

Chromatographic

Extraction Chromatography. (*Journal of Chromatography* Library, Volume 2.) Edited by T. Braun and G. Ghersini. Pp. xvii+566. (Elsevier Scientific: Amsterdam, London and New York, 1975.) Dfl. 130; $54.25.

THE title of this book will be unfamiliar to most western practitioners of chromatographic separation methods. It is defined in chapter 1 as the reversed-phase partition chromatographic separation of ions usually, although not necessarily, using systems which greatly favour partition into the stationary phase. Various means, including complexing and chelating agents as well as physical partition, are used to achieve this end. The separations described in this book are exclusively inorganic, although this does not seem to be intrinsic in the method.

I personally feel that this nomenclature is unfortunate in that it does not give due emphasis to the distribution between two phases, which is the essential mechanism of all chromatographic processes. It has arisen, however, from the extensive use of liquid–liquid extraction in the separation of the products of nuclear fission, the chromatographic applications having followed later. In so far as the term extraction chromatography is restricted to those inorganic chromatographic separations, in which an ion is converted into a non-ionic complex which is extracted preferentially into a non-aqueous stationary phase; it is probably an acceptable innovation.

Within these limits this collaborative work succeeds admirably. After introductory chapters on the theoretical aspects and the correlation between chromatographic and liquid–liquid partition results, three excellent chapters describe experimental methods (which are necessarily rather elaborate in all reversed-phase partition chromatographic techniques), stationary phases (in considerable detail) and support phases. The last by Katykhin is outstanding and will be very valuable to organic chemists and biochemists as well as those concerned exclusively with inorganic separations. This chapter contains a wealth of tabulated material on all the commonly used support phases, including physical properties such as density, surface area, total porosity, average pore diameter, capacity for typical organic stationary phases and HETP values for a wide range of chromatographic systems. International suppliers, including those in east European countries, are also tabulated. To my knowledge no such extensive compilation has appeared elsewhere. Katykhin also discusses the pretreatment of the support materials and column packing. He also includes a description of the preparation of sintered porous PTFE blocks which can be moulded into any required shape, and can be used repeatedly in a variety of reversed-phase chromatographic systems, including annular blocks for continuous chromatography.

The remainder of the book deals with special applications of extraction chromatography to specific inorganic ionic groups. Stronski deals with the separation of radionuclides, and shows that in the majority of cases a difference of unity in atomic number suffices for complete separation. Separations of the individual elements are also usually possible in most vertical columns of the periodic table, for example, Li, Na, K, Rb and Cs; Ca, Sr and Ba; Au, Pt and Pd; and the halides, F, Cl, Br and I. Complete separations in the IVth, Vth and VIth groups, however, are still difficult. Separations of the actinides and lanthanide elements are described in chapters by Muller and Siekierski.

In summary it seems that good separations of most pairs of inorganic ions can now be achieved simply and effectively by extraction chromatography both on the analytical and preparative scales. The method is particularly suitable for highly radioactive elements, as preparative separations can readily be carried out by simple remote control in well-shielded enclosures, whereas analytical separations can be carried out at such high dilutions that special precautions are usually unnecessary.

This book provides an excellent and complete coverage of the whole field of the separation of inorganic ions by column, thin-layer and paper chromatographic methods, and as such it is highly recommended.

C. J. O. R. Morris

Insect miscellany

Insects, Science and Society. (Proceedings of a Symposium held at Cornell University, Ithaca, New York, October 14–15, 1974.) Edited by David Pimentel. (Academic: New York and London, July 1975.) $15; £7.20.

THE symposium on which this book is based was held in Cornell University in October 1974 to celebrate the centenary of the teaching of entomology at Cornell—indeed, in the United States—coupled with the name of the founding father John Henry Comstock, who gave the first course of lectures while still an undergraduate. The well known achievements of Comstock are briefly reviewed by E. H. Smith. D. Pimentel gives a general introduction on insects and crop production in the US, and J. J. McKelvey a colourful picture of 'insects and man'. E. O. Wilson contributes a brilliant short essay on the evolution of insect societies, in which their potential for studying the nature of sociality is stressed.

R. D. Alexander gives a lengthy account of chorussing behaviour in Orthoptera and cicadas, which is largely a re-interpretation of the author's earlier observations, the stress now being laid on the selective advantage secured by individual males in the competitive search for a mate, rather than on the advantage for the group. W. L. Roelofs reviews his own researches on the red-banded tortrix (leafroller) as an example of the physiological properties of pheromones and of their potential use in monitoring populations and perhaps in control by trapping or mating disruption. In a stimulating essay J. S. Kennedy argues persuasively for the view that all migration (and that includes 'dispersal') in insects involves a behavioural response, an essential component of which is the suppression of reproductive ('vegetative') activity and its temporary substitution by active locomotion, with or without passive transport by the elements. M. D. Pathak reviews the methods and results of plant breeding for resistance to insect pests as illustrated by the wide ranging studies on rice.

According to a valuable contribution by T. R. E. Southwood the competing models of population dynamics at varying densities all contain elements of the truth, and that an approximation to real life can be obtained by integrating these models into a single framework which also takes account of habitat stability. As P. S. Messenger points out in a paper on parasites, predators and population dynamics in California, that even in highly developed monocultures the complex of insect species is very large and general predators are a major element in integrated control schemes. W. Klassen gives an account of the planning and administration of the insect control programmes as organised by the US Department of Agriculture, in which it is claimed that increasing stress is being placed on 'integrated control'.

The final article, by L. D. Newsom, deals with 'pest management' (synonymous with integrated control)—the standard procedure of the entomologists of the nineteenth century, which he illustrates vividly in relation to cotton and soya bean culture in the US. There seems little doubt that the future lies with these ecological procedures. But they require skilled and imaginative entomologists; they are vigorously opposed by the salesmen of the chemical industry whose approach appeals more to the grower; and they still lack administrative encouragement. They do, however, have some enthusiasts on their side.

Although not closely integrated, and although reproduced in offset typescript, this book contains a high proportion of valuable articles.

V. B. Wigglesworth

Stellar essays

Problems in Stellar Atmospheres and Envelopes. Edited by B. Baschek, W. H. Kegel and G. Traving. Pp. xix+375. (Springer: Berlin and New York, 1974.) DM48; $20.70.

THE occasion of the seventieth birthday of one of the world's most distinguished authorities on the theory of stellar atmospheres and the quantitative analysis of spectral lines—Professor Albrecht Unsöld of Kiel University—has been marked by the production of this *Festschrift* to which eleven of his former pupils have contributed review articles on stellar atmospheres and related topics, expressing at the same time the hope that others will find them interesting and stimulating.

This hope is certainly fulfilled by the great majority of the articles, in which the authors give their personal viewpoint on several topics of current interest in stellar astrophysics. Classical problems relating to the structure of stellar atmospheres are discussed by D. Labs, who gives a convincing defence of the solar irradiance tables published by Labs and Neckel in 1968 and 1970, and by Mrs E. Böhm-Vitense who uses a grid of model stellar atmospheres in radiative and local thermodynamic equilibrium to predict continuous spectra and colour indices as a function of temperature, luminosity and chemical composition. From a less classical point of view, G. Traving discusses the formation of spectral lines in turbulent media and the physical significance of the controversial empirical parameter known as "microturbulence". Moving out from "atmospheres" to "envelopes", W. H. Kegel describes the theory of maser action in OH and H_2O emission sources, while L. Oster gives a rather entertaining historical review of radio emission from stars and its significance —a subject that has been close to Unsöld's heart since the 1950s— and K. H. Böhm describes physical conditions in the rather mysterious Herbig–Haro objects and in the related nebulae surrounding T Tauri stars, which may result from shock-heating by the impact of a stellar wind on the intracloud medium.

The remaining articles concern the application of stellar atmosphere theory to abundances of elements and other problems in stellar internal structure and evolution. K. Hunger discusses the helium stars, concluding that some of the intermediate variety (as well as the extreme ones) achieve this state by nuclear evolution, whereas others may be affected by atmospheric diffusion. Further abundance anomalies in hot stars (mainly with weak helium lines) are lucidly described by B. Baschek, together with the many mechanisms that have been proposed to account for them, among which the diffusion hypothesis of G. Michaud is generally the most successful, although other mechanisms may also be involved. Diffusion is also clearly important in white dwarfs, which are discussed in a useful review by V. Weidemann that covers their role in galactic evolution as well as their atmospheric properties. K. Kodaira describes the spectral analysis of A-type horizontal-branch stars which is of interest both as a source of information on the chemical composition of old objects and as a means of determining the mass–luminosity ratio, which can then be compared with predictions from evolution and pulsation theories; and D. Reimers discusses the rate of mass loss from red giants on the basis of circumstellar absorption-line observations, deriving an empirical formula which shows that observed mass-loss rates are sufficient to satisfy evolutionary requirements for stars having less than twice the mass of the Sun.

Altogether this book is a worthy tribute to its illustrious dedicatee and a welcome addition to the astronomical review literature.

Bernard Pagel

Imaginative reflections

The Lives of a Cell: Notes of a Biology Watcher. By Lewis Thomas. Pp. 153. (Viking: New York; Garnstone; London, November 1975.) $6.95; £3.50.

FOR once the publisher's blurb is correct; this is an extraordinary book. At first glance this is not apparent. The book is a collection of essays, 29 of them, each no more than about five pages long, reprinted from issues of the *New England Journal of Medicine* from 1971 to 1973. The author is a distinguished clinician, but the essays are not about medicine (except incidentally); most of them are imaginative reflections about societies of insects and men. The best way to convey the flavour of Thomas' book is to say that he looks with wonder and understanding at the behaviour of some simple organisms—in which he includes mitochondria, chloroplasts, bacteria, ants and termites—and his wonder leads him to speculate about the behaviour of man. This theme could be a recipe for sentimental teleology—a sort of latter-day Maeterlinck. But Dr Thomas is too good a biologist to fall into that trap. He knows that to impute even the rudiments of human intelligence to insect communities is nonsense; social insects have no choice but to build nests or hives, to store food, to cultivate fungi, to live in symbiosis with other creatures; all under inflexible genetic compulsion. But (he asks) are there not also examples of genetically coded behaviour in men and communities of men? And it is his speculations about this question which make this book such delightful reading. I use the word 'delightful' deliberately; for the book contains few facts which are not familiar to biologists, and it does not weave these facts into logic, it spins speculations out of them. The essays are little flights of imagination, written with charm and wit. But they are anything but trivial. In practically every essay something familiar is suddenly put into a fresh light, and it looks quite different; and this, of course, is precisely how creative science is achieved. Dr Thomas has a flair for 'thinking otherwise' about familiar things.

For example, he includes mitochondria as 'organisms', for one of his themes is the prevalence of symbiosis in the whole spectrum of nature from termites to men. Mitochondria, he explains, "replicate in their own fashion, privately, with their own DNA and RNA quite different from ours." They are "stable and responsible lodgers" in the human body. Symbiosis is a symbol of the interdependence of living things, part of the process which constructed metazoans from single cells, communities from metazoans, and ecosystems from communities. He calls as witness the symbiotic service which the protozoan *Myxotricha paradoxa* gives to the termite community, providing the enzyme to break down cellulose.

Another theme of these essays starts with the question asked two generations ago by W. M. Wheeler: "To what extent can insect communities be regarded as organisms?" (It was Hegel who asked a homologous question about the State.) A familiar enough idea, but Dr Thomas illuminates it from a fresh direction:

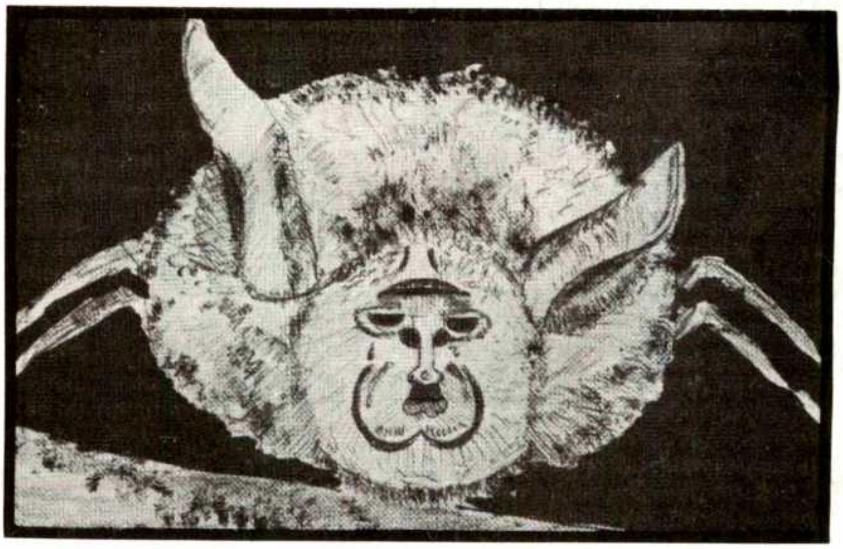
> "Viewed from a suitable height, the aggregating clusters of medical scientists in the bright sunlight . . . at Atlantic City, swarmed there from everywhere for the annual meetings, have the look of assemblages of social insects. There is the same vibrating, ionic movement . . . the darting back and forth of jerky individuals to touch antennae and exchange small bits of information . . ."

And what are the scientists doing? They "are linked in circuits for the storage, processing, and retrieval of information." What societies of termites do with cellulose, societies of men do with information, rearranging it against randomness into such patterns as quantum mechanics, immunology, genetics. And so Dr Thomas sees an analogy between the termite team (ineffective unless there is a quorum of termites), building something beyond the conception of any of the individual builders, and the invisible college of scientists, piecing together fragments of information into ordered patterns of understanding. There even seems to a similar compulsion:

> "Scientists at work have the look of creatures following genetic instructions; they seem to be under the influence of a deeply placed human instinct. They are, despite their efforts at dignity, rather like young animals engaged in savage play."

As a solution appears they become agitated, as bees do in a disturbed hive, and there "suddenly emerges, with the purity of a slow phrase of music, a single new piece of truth about nature."

Fanciful? Yes, it is; but it is this sort of fancy which acts as a catalyst for hard science. As A. N. Whitehead wrote: "It is more important that a proposition be interesting than that it be true." And Dr Thomas' theme is intensely interesting, for he goes on to suggest that there is one activity of human communities which has an uncanny resemblance to the genetically coded activities of social insects, namely the development and evolution of language. Chomsky has postulated that language must be a biological property of the human mind. The capacity to apprehend syntax is genetically coded. Language grows, changes, evolves, independently of any control or planning by individuals or committees or governments. All of us unconsciously and inevitably contribute to its growth and metamorphosis, with as little understanding of how we do it as a termite has of the architecture of its hill. Language, Dr Thomas says, is "the most compulsively collective, genetically programmed, species-specific, and autonomic of all the things we do, and



Greater Horshoe Bat with minute eyes on either side of the nose. The curved portion below the nose and surrounding it represents the nose-leaf, which directs sound waves emitted through the nose during flight. Taken from *Vision in the Animal World* by R. H. Smythe. (Macmillan: London and Basingstoke, 1975.) £6.95.

we are infallible at it." Thus, although an anthropomorphic view of termites is undoubtedly bad science, a termitomorphic view of some of man's activities may be illuminating.

There are many other refreshing comparisons between human and animal behaviour in these essays. For instance, a great deal is known about olfactory sensing among animals and insects; but smells play no part in recognition or signalling between human beings. Because of this deficiency (writes Dr Thomas) "we feel somehow inferior and left out of things." Perhaps this sense has atrophied among humans; but the specific smells are still there, for a tracker dog can follow the trail of a man across the tracks of dozens of other persons. A promising line of research here (writes Dr Thomas) for an Institute of Human Fragrance; for if smells are so specific that dogs can distinguish them, have they any link with the equally specific immunological characters in man?

Readers of *Nature*, accustomed to the severe austerity of prose in which a scientific communication has to be made if it is to be credible, may well ask what relevance these whimsical essays have for them. I think they have considerable relevance, and this is why I think so. In the 1970s pure scientific research is on the defensive. Not only is there the first anti-science movement for over a century; there is also, and more ominous, an air of scepticism about the value of pure science on the part of the Establishment. Research Councils are considering the cost-benefit values of their support for some lines of work. Government-sponsored science has now to be regarded as a sort of trade-relationship between 'customers' (who often do not know how to ask the right questions) and 'contractors' (who are paid to answer the questions even if they are not the right ones). In these circumstances it is essential that the public should understand the value to civilisation of encouraging sheer exuberant curiosity about nature. They will not understand this unless they understand what the scientific attitude to nature is like. The end results of scientific research are well popularised in books, films, and TV programmes; but the public know very little about the conception of scientific ideas, the leap of imagination necessary to ask the seminal questions to which research provides the answers. The future patronage of science by the public may therefore depend on a fresh kind of popularisation. If I had to recommend to a non-scientist a book which gives a vivid impression of how scientific ideas are born, this is the book I would recommend.

Eric Ashby

Problems and progress in theoretical physics

Quantum Gravity: An Oxford Symposium. Edited by C. J. Isham, R. Penrose and D. W. Sciama. Pp. 605. (Clarendon: Oxford; Oxford University: London, July 1975.) £10.50.

The first quarter of this century witnessed two major revolutions in fundamental theoretical physics: quantum theory and relativity. As we enter the last quarter of the century, these two cornerstones of modern physical science are still irreconciled.

Quantum theory was originally developed as a theory of matter, whereas relativity is a description of the structure of space-time and its relationship to gravitation. The latter development of the quantum theory of fields, however, with its spectacular successes in describing electrodynamic phenomena, naturally prompted investigation to determine whether these successes could be repeated for the gravitational field. The only theory of gravity so far tried for quantisation in any seriousness is Einstein's general relativity (and minor modifications thereto), an enterprise which has encountered extraordinarily complicated technical problems, demanding great dedication and courage from would-be quantisers. These difficulties, together with some fundamental problems of principle, have resulted in a whole range of well-tried approaches, all of which have so far failed to yield a satisfactory quantum theory of gravity. Many practitioners, disillusioned with the slow progress of these "frontal assaults", have advocated nothing less than a rejection of the direct use of either general relativity or quantum theory, and propose instead intriguing new principles at a more elementary level of nature. In direct contrast, another popular "second-best" effort has been to try to first cope with the quantum theory of better understood fields which are located in the curved space-time of general relativity, that is, quantum matter in classical gravitational fields.

A whole range of such philosophies and techniques appear in this impressive book. The text, apart from an article by S. Deser, is based on a symposium given at the Rutherford Laboratory in February 1974. There are nine chapters, and although each is by a different author, the organisation and level of presentation are sufficiently consistent to make the book a very useful collection of topics.

The symposium itself was a memorable occasion, because it simultaneously provided a good deal of pessimistic prognosis about achieving a proper quantum gravity using the more traditional techniques, together with the first public announcement of an extraordinary new result about black holes by Stephen Hawking. There is no doubt that this development, which predicts that black holes create particles and "evaporate" away, has provided a great stimulus for research into quantum field theory in curved space.

Hawking's explanatory article is accompanied by one of the most concise and elegant accounts so far of the theory of twistors, presented in two chapters by Roger Penrose and his former student, George Sparling. Penrose's twistor formalism provides by far the most developed and promising possibility of a completely new approach to the structure of space-time and matter.

The more traditional techniques are dealt with by M. J. Duff, with a clear account of covariant quantisation, S. Deser discussing recent Feynman graph calculations and A. Salam, who outlines some results from his long programme of treating gravity from a particle physicist's viewpoint. The main conclusion to emerge from these discussions is the impasse of non-renormalisability; when matter is present, the divergences which always occur in quantum field theory cannot be isolated for the gravitational field in a systematic fashion.

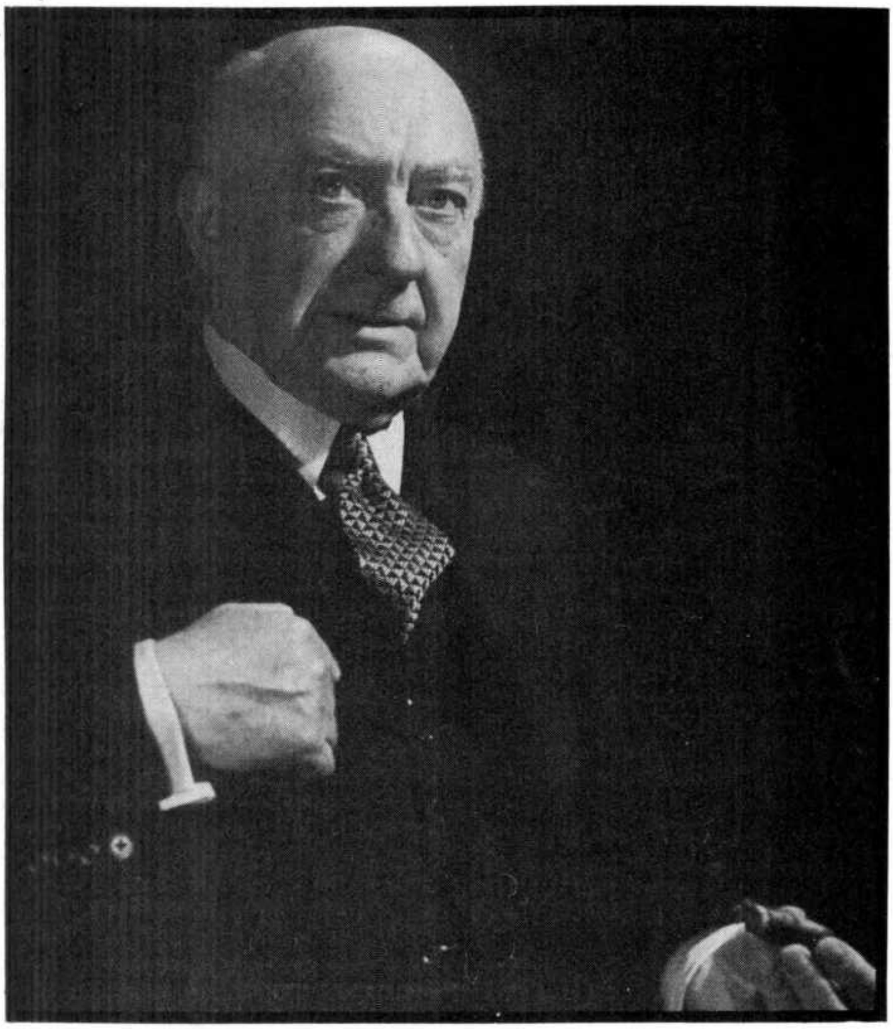
The book also contains a well-written account of some aspects of quantum cosmology—the slightly bizarre technique of quantising the whole universe as a simple mechanical system—by M. A. H. MacCallum. The final chapter carries the unmistakable stamp of J. A. Wheeler in a wide-ranging assessment of the subject, complete with philosophical perspectives and intriguing illustrations. This chapter, entitled "Is Physics Legislated by Cosmogony", is jointly written by Wheeler and his student, C. M. Patton.

No book of this type could be complete without a good review of the whole subject. A useful and very readable account is given at the beginning by an acknowledged quantum gravity expositor and contributor, C. J. Isham.

For the first time the researcher has available in a single volume a broad range of material, edited by three experts, in this most challenging and difficult area of theoretical physics. It is to be recommended to all departments with an interest in cosmology and astrophysics, and more generally to anyone concerned about the really fundamental aspects of physics.

P. C. W. Davies

Portrait of an industry



Baron McGowan of Ardeer. Courtesy Douglas Glass

Imperial Chemical Industries: A History. By W. J. Reader. Vol. 1: *The Forerunners, 1870–1926.* Pp. xvi+563+38 plates. £7.50 net. Vol. 2: *The First Quarter-Century, 1926–1952.* Pp. xvii+569+41 plates. £18.50. (Oxford University Press: London and New York, 1970 and 1975.)

IN the first volume of this work Dr Reader traced the histories of the four major companies—Brunner Mond, Nobel Industries, United Alkali, and British Dyestuffs Corporation—which in 1926 participated in the biggest merger in Britain, up to that time, to form Imperial Chemical Industries. He gave convincing evidence of his ability to digest an immense amount of detailed information and to present it, very fully annotated, in a readable form. Reader's treatise is, however, essentially a business history— with its emphasis on finance, commercial strategy, and trade practices—and the reader with an interest in the history of technology is less adequately catered for, though he will find the cited references helpful. He will learn more about the consequences of technological advances than about their causes and natures. There is, for example, rather little about the basic problems of high-pressure technology—fundamental to ammonia synthesis—and how they were overcome. Nor do we learn much of the technical problems of the switch from coal to oil as a raw material after the war. In a history of a pre-eminently science-based industry, where decisions are determined as much by technological feasibility as by commercial acumen, that is a little disappointing.

In the latest volume Dr Reader expounds his main theme, the evolution of ICI during the first 25 years of its existence. As before, he has had unrestricted access to the company's archives and he has written very frankly of failure as well as success, of the weaknesses as well as the strengths of the individuals concerned. As volume 1 relates, the immediate reason for the formation of ICI was McGowan's clear recognition that the existing units of the British chemical industry were too small to compete effectively with the powerful IG Farbenindustrie formed in November 1925. The merger was very hastily cobbled together—literally as Brunner Mond were on the point of allying themselves formally with IG—and in the early years much effort had necessarily to be expended simply on welding four very disparate entities into a unity which some of them did not really want.

Although a distinguished board was appointed, power from the outset lay with Sir Alfred Mond (later Lord Melchett) and Harry McGowan. After the former's death in 1930 the board elected McGowan chairman and managing director. This unwise dual appointment gave him extraordinary power, of which he availed himself to the full: from 1930 onwards he ruled ICI absolutely. Not until 1937, when he was away in Australia, was his power challenged by the board. Then, just as it seemed that success was within their grasp, what Reader calls "The Barons' Revolt" fizzled out—possibly because of the illness, at the crucial moment, of the second Lord Melchett.

In the early years, ICI's commercial policy was wholly unadventurous. McGowan was a firm believer in limiting production, sharing markets, and—above all—securing to ICI the market in the Empire which its name embodied. To him and his colleagues the Empire was enduring and virtually indestructible: over the years it would provide a reliable and growing market from which all foreign rivals could be excluded, if necessary by making agreements not to enter their territories. It is very reminiscent of Pope Alexander's Bull of 1493 dividing the New World between Spain and Portugal. The idea of competition was positively repugnant. As late as 1941, McGowan wrote that he did not accept "the theory . . . that competition is essential to efficiency". The policy was indeed not unsuccessful at that time but it led to some very serious complications, as, for example, in the case of the Dupont agreement. The United States had very different views of the nature of free enterprise.

It was not the only error. Another was in the great fertiliser complex built at Billingham. Technologically, it was a great achievement, but it proved a commercial disaster when markets could be found for only a fraction of its capacity. Reader brings out one reason for that, other than the slump, very clearly: marketing was defective because ICI failed to recognise the crucial importance of the men concerned and accord them the necessary status. Production methods were good, but it seemed that selling the products —especially to the public as opposed to other manufacturers—was too ungentlemanly to be taken seriously.

The half-hearted approach to profitability was not without national advantage, even if not to the shareholder. There is no doubt whatever, that, over the period here dealt with, ICI was actuated by a high sense of public responsibility. At no time was this better expressed than during the war when the company's resources, not only in production but in very able manpower, was put at the nation's disposal to an extent far beyond the call of duty, with profit as a secondary consideration.

Failures there may have been, but there were resounding successes too, among them polythene and Terylene. Apart from such highlights, however, the substance of the tale told here is one of solid, if unspectacular, achievement over a vast industrial field covering non-ferrous metals, pharmaceuticals, dyes, paints, fertilisers, plastics, fibres, and many other products.

There is one field of ICI's activities deserving greater mention than is given by Reader, and that is the field of publicity and public relations. The company, under the late Sidney Rogerson, pursued an enlightened policy which set new standards. Works were commissioned not only from established artists but also from younger aspirants yet to make their name. Equally high standards were set for the literary style. Older readers will recall such outstanding series of advertisements as 'Ancestors of an Industry', 'Equipment of an Industry', and 'Portraits of an Industry' (from the last of which, incidentally, eight of the illustrations of the book are taken, though the source is not mentioned). Another series, the 'Colours of Chivalry', was ultimately made available as a book which is now a collectors' piece. No mention is made either of *Endeavour*, an international scientific review in several languages, which was started during the war and afterwards established itself as an accepted part of the world's scientific literature. Equally, no reason of modesty should have prevented much fuller mention of the company's enlightened policy towards the universities.

The Postdoctoral Fellowship Scheme, begun in 1944 and only just terminating, gave a start in professional life to well over a thousand able young scientists. Many of them have gone on to outstanding careers in science, and they include two Nobel Laureates.

It is, of course, very easy to criticise a work of this kind on points of detail. Unquestionably, however, this is a masterly account of the formative years of a vast supranational company. Others might have done it rather differently, but it would be difficult indeed to have done it better.

T. I. Williams

Comprehensive biochemistry

Medizinische Biochemie: Lehrbuch für Studierende und Ärzte. 6., Völlig neu bearbeitete Auflage. By S. M. Rapoport. Pp. xxvii+1023+14 tafeln. (Volk und Gesundheit: Berlin, Jena 1975.) DM53.

Comprehensive Biochemistry. Vol. 31: *A History of Biochemistry.* Part 3: *History of the Identification of the Sources of Free Energy in Organisms.* By Marcel Florkin. Pp. xx+475 (plates 64–120). (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.130; $54.25.

Of its kind Rapoport's is one of the best German textbooks of biochemistry for medical students. The book is too detailed for mere preparation for preclinical examinations but is very suitable for keen students, especially those in their clinical years and beyond, who wish to read about the biochemical background of diseases. The material is well selected with special reference to the practice of medicine. The fact that the book has reached its sixth edition since it was first published in 1962 is a measure of its popularity. The new edition is brought up to date with the help of several close collaborators.

What makes the book attractive is its coherence and readability. This is achieved by linking general comments, often of a highly original character, and by avoiding the mere assembly of factual information. The interest of the medical student and medical graduate is maintained by continually relating the biochemical information to normal and pathological organ function. Selected references for advanced reading are given. More frequently than is usual in western treatises are references to Russian investigators and to the importance of their contributions to the progress of the subject.

The text is occasionally interspersed with references to Marxist philosophy—for example, by quoting (on page 35) Engels' formulation of the nature of life: "Life is the manner of existence of protein, the chief property of protein being the continuous exchanges of material with the external environment; the cessation of these exchanges causes the decomposition of proteins". This statement is taken to relate to the dynamic state of proteins, predicted in this passage by Engels many decades before it was experimentally established.

Florkin's work is the first comprehensive effort since Lieben's *Geschichte der Physiologischen Chemie* (1935) covering a field which has grown to vast dimensions in recent decades.

This is the second of four volumes planned as the last section of the 33 volumes of the reference work *Comprehensive Biochemistry*, edited by Marcel Florkin and Elmer Stotz. Florkin, who is writing all the four volumes, is well qualified to undertake this difficult task as he is a biochemist who has researched in many areas, especially in comparative biochemistry and has had the unusual distinction of having been president of the International Union of Biochemistry—an indication of his standing as a biochemist, president of the International Academy for the History of Medicine and vice-president of the International Academy of the History of Science, on account of several major publications on historical aspects of medicine and science.

The present volume is concerned with the history of the sources of energy in living organisms, that is, fermentations, glycolysis, biological oxidations and photosynthesis. The narrative is detailed, giving the key references to the original papers, quoting literally many telling passages, and reporting the story of the complex ways by which the edifice of knowledge gradually took shape—a process which involved not only construction but a fair amount of demolition. Individual scientific publications often give an impression of a continued series of successful experiments because all the disappointing failures (although they may have been essential and unavoidable steps) have been omitted from the final presentation. At a different level this is also true for the history of scientific developments. Florkin illustrates this by relating many of the aberrations which are part and parcel of the steady progress of science.

Students of science are nowadays often disinclined to concern themselves with the historical development of their subjects. They feel that there is so much to read and to learn about the present state of affairs that there is no spare time for concerning themselves with how the knowledge was arrived at, and in particular with reading about aberrations which led to dead ends. Understandable though this attitude may be, for the proper appreciation of scientific knowledge it is essential, especially for those engaged on research, to know how knowledge was arrived at.

The way in which Florkin writes makes it pleasant and easy to get glimpses of the historical background of present knowledge. I feel confident that the book will be enjoyed by many readers, and as a source-book it will remain a standard reference work for a long time to come.

H. A. Krebs

Common sense, wa hae

The Triumph of Truth *by Joshua Reynolds, 1774. The figure on the right portrays James Beattie (1735-1803) holding his* Essay on Truth. *The Angel of Justice is thrusting down the head of Voltaire. The head in the foreground was believed by David Hume to be his: that in the left corner was said to be of Edward Gibbon.*

Scottish Philosophy and British Physics, 1750–1880: A Study in the Foundations of the Victorian Scientific Style. By Richard Olson. Pp. vii+349. (Princeton University: Princeton, New Jersey, and London, August 1975.) £9.20.

"WHERE we try to find models or analogies, they are quite content with laws", so wrote H. G. J. Moseley in his diary for June 1914 after meeting the French physicist Urbain. And if "pictorial thinking" has been a major and distinguishing characteristic of British physics, it is tempting to speculate on how it came about. The interesting thesis of Dr Olson is that it started with the 18th century Philosophical Society of Aberdeen (also known as the Wise Club) whose two most influential members were Thomas Reid, Professor of Philosophy at King's College, and James Beattie, Professor of Moral Philosophy at Marischal College.

The Society was the cradle of Scottish Common Sense Philosophy, so little known today that it has no mention in Bertrand Russell's *History of Western Philosophy*. It arose as a reaction against the atheistic scepticism of David Hume (to whom in fact it owed a good deal) and the materialism of thinkers such as Voltaire and Joseph Priestley. The Aberdeen group, in their efforts to defend moderate Scottish Presbyterianism against the rationalist challenge, had encountered the difficulty that many of us currently face in battling with the prospect of anarchy based on specious rationalism: the latter can lead to conclusions which in Reid's words contradict "certain principles which the constitution of our nature leads us to believe, and which we are under a necessity to take for granted in the common concerns of life". These are moral principles which can be no more proved than the axioms of Euclid and yet which are correspondingly essential as the foundations for a framework of morality.

This seems to be a remote base from which to trace the rise of pictorial thinking in British physics, but it is what Dr Olson sets out to do:

> "The basic aim of this book is to present and to establish the probability of a hypothesis regarding the development of the exact sciences—that is, that many of the important characteristics of the exact sciences in Scotland, and subsequently throughout Britain can be accounted for by the fact that Scottish scientists adopted a particular set of methodological and epistemological attitudes which were clearly articulated by a group of moral philosophers collectively known as the Common Sense School".

Perhaps the most suggestive argument is that with its reliance on common sense plus a few moral axioms, Scottish philosophy had greater faith in the pure "geometry of visibles", proving results by Euclidean arguments, than in analysis, where the algebra of infinite series was known to be dangerous, and where some of the entities such as the square root of minus one had no common-sense physical interpretation. Certainly, there was strong interaction in the Scottish universities between the moral and the natural philosophers (Beattie was in fact appointed to the Chair of Natural Philosophy but exchanged it the same day for that in Moral Philosophy) and undergraduates were exposed to both influences in the traditional Scottish degree.

And there was plenty of good teaching to be had: Reid held with Bacon that the stages of human knowledge were "like steps of a ladder" in which it would be "fruitless to skip directly from bottom to top"—a lesson often ignored today with disastrous results in school teaching. We find John Playfair distinguishing between hypothesis and theory:

> "Hypotheses are explanations which have no evidence independent of their ability to account for the phenomena in question while theories are founded on facts known independently of the phenomena to be accounted for".

And William Hamilton extended Occam's Razor into the Principle of Parsimony:

> "neither *more* nor *more onerous* causes are to be assumed than are necessary to account for the phenomena".

Again, Hamilton spoke of

> "the highest faculty of the mind—that of tracing the analogy of unconnected observations of evolving from the multitude of particular facts a common principle, the detection of which might recall them from confusion to system, from incomprehensibility to science".

Hamilton's colleague at Edinburgh, J. D. Forbes (of the bar and glaciers, and the Chair of Natural Philosophy) wrote

> "The importance of analogies in science has not, perhaps, been sufficiently insisted upon by writers on the methods of philosophizing. A clear perception of connexion has been by far the most fertile source of discovery".

Dr Olson ascribes the demise of the Scottish common-sense tradition to Forbes because his advocacy of greater specialisation in science at the undergraduate stage dethroned moral philosophy from its paramount place in Scottish education. But he as well as Hamilton influenced their pupil, Clerk Maxwell, who all through his life was fascinated by analogy—for example asking the question in a postgraduate essay "Are there real analogies in nature?", and repeatedly stressing the fruitful function of analogy throughout his papers and addresses. As a useful to alert potential readers to culmination, his Electromagnetic Theory showed him to be the greatest of all masters of analogical thinking.

This may be the point at which to mention some very minor blemishes in the book, for Maxwell spent his boyhood not in Aberdeenshire (p. 287) but Kirkcudbrightshire. Melloni's Christian name was Macedonio not Marcello (p. 227) and Poynting's initials were J. H. not M. H. (p. 328), and there are occasional mispellings.

Whether through common-sense Philosophy or not, the Scottish contribution to science up to 1870 was formidable. Besides those of Forbes and Maxwell it is easy to recall many names: Black, Brewster, Brown, Graham, Hutton, Leslie, Lyell, Nicol, Rankine, Ritchie, Robison, Tait, Waterston, Watt, the Gregorys, the Playfairs, and the Thomsons. Quite possibly there was more to it than the common-sense tradition alone: in Britain generally, religious dissent also seems to have been a factor, and the relative decline of the Scottish universities after 1870 was probably far less due to Forbes than to the revival of Oxford and Cambridge, with the abolition in 1871 of religious tests for university entrants. But Dr Olson has produced a book of remarkable interest, well worthy of detailed study by everyone interested in the philosophy of science. **R. V. Jones**

Earth studies

The Earth's Density. By K. E. Bullen. Pp. xiii+420. (Chapman and Hall: London, 1975.) £12.90.

THIS book gives an historical account of developments concerning the variation of density within the Earth. The first five chapters are of an introductory nature covering noted ancient investigations, the determination of the Earth's mean density, spherical harmonics, theory of the Earth's gravitational attraction and the figure and moment of inertia of the Earth. The sixth chapter covers early models of the Earth's density variation including the Legendre–Laplace density law and the Williamson–Adams equation. The meat of the book (chapters 7–17) deals with seismic wave transmission and presents contributions from studies of P and S waves, surface waves, and free oscillations. The penultimate chapter deals with optimum and standard Earth models and the final chapter contains a discussion of the densities of the other planets.

The author, a mathematician, has been foremost in developing Earth models of the classical type based on the interpretation of travel time curves, and chapters 9 and 10 provide a resumé of the Bullen Earth Models of types A and B. Other procedures, such as the Monte Carlo technique and general inversion, are also discussed but somewhat unenthusiastically. The reader will naturally turn to the chapter on optimum and standard Earth models and there he may be disappointed. It seems we still do not have a standard Earth model and the Monte Carlo and general inversion models are treated with scepticism. Bullen's choice of the best model is shown in Fig. 16.1 but it is not clear which model this is and no error bars are shown; it is presumably a combination of Bullen–Haddon models. It is unfortunate that the big conclusion is somewhat obscure as the rest of the book is so beautifully meticulous.

Throughout, the Earth is assumed to be spherically symmetrical, and though lateral variations of density are frequently discussed they are usually dismissed as minor. This is a pity, as with more precise data (using WWSSN and seismic arrays) it is becoming apparent that the Earth is far from spherically symmetrical and that there is considerable local variation in travel time curves. Most of the work described dates from a time before the acceptance of sea floor spreading and 'rapid' plate movements. The outermost layers of the Earth are very mobile and we have few clues as to what causes the mobility, so studies of lateral variations of density are clearly of great importance. We will need to know how big they are and how deep they extend. With that kind of information it may become possible to develop theories to explain plate movements and the earthquakes associated with them. In the meantime, Bullen's book gives us all the hard facts of classical seismology with the warning that they mustn't be forgotten as the lesson for the day. **R. W. Girdler**

The Viscosity of the Earth's Mantle. By Lawrence M. Cathles III. Pp. 386. (Princeton University: Princeton and London, 1975.) £13.10.

THE viscosity of the Earth's mantle is obviously important, not least because of the restrictions that its absolute value and variations with depth place on possible models of mantle convection. Nevertheless, it comes as something of a shock to learn that it can form the subject of a complete, long book. The shock is magnified (or perhaps diminished, depending on one's point of view) by the discovery that, although previous literature is briefly reviewed where appropriate, this is not a textbook but an original work. In short, it is really a long scientific paper which happens to be bound between hard covers. The normal rules of book reviewing therefore hardly apply; proper criticism of this work will only come from other workers in the field over a period of years, rather than weeks or months.

In the meantime, it would seem most Cathles' chief conclusions, especially as one or two of them, if not actually controversial, certainly defy the consensus. The study is based on the assumption that the Earth is a self-gravitating viscoelastic solid; and in the first part of the book the mathematical techniques required to model the isostatic adjustment of such a body are developed at length. The theoretical foundations thus acquired are then combined and compared with geological data on the way the Earth responded to Pleistocene glacial–water load redistributions to deduce mantle viscosity and its variations.

The 'average' Earth model thus constructed has a lithosphere which may be regarded as an elastic shell with effectively infinite viscosity and thickness varying between 70 and 150 km. Immediately below the lithosphere is a low viscosity layer (or 'channel') with an average thickness of about 75 km and a viscosity of about 4×10^{20} poise. The evidence for such a channel is widely distributed geographically, although both the thickness and viscosity of the channel probably vary considerably from place to place. Between depths of 75 and 1,000 km measured from the top of the low viscosity channel, the mantle (defined as the 'upper mantle') has a viscosity of $1.0\pm0.1\times10^{22}$ poise—a conclusion based largely on the uplift of Fennoscandia which has a further 30–50 m to rise.

Most interest, however, is likely to centre on the rest of the mantle (the 'lower mantle') which also has a viscosity of about 10^{22} poise, although at $0.9\pm0.2\times10^{22}$ poise it might be very slightly lower than that of the upper mantle. Many (but not all) workers believe that the lower mantle has a much higher viscosity and that mantle flow is limited to the asthenosphere. But a viscosity of about 10^{22} poise throughout permits full-mantle convection. Moreover, if full-mantle convection is occurring, its main function must be to transport heat from the Earth's interior into space (by way of lithospheric conduction), which can be done effectively only if the upwelling convective limbs are thin. In other words, Cathles' analysis supports mantle plumes, whether cylindrical or sheet-like. Following Morgan, the plumes may then be envisaged as spreading out at the top into the low viscosity channel, with at least a part of the convective return flow occurring uniformly through the mantle as a whole.

Last, but not least, Cathles' model assumes right from the start that viscosity is Newtonian throughout the mantle. This turns out to be entirely consistent with glacial uplift data.

Peter J. Smith

SCIENCE COLLEGE

Pantheism in modern dress

ALISTER HARDY is not the only distinguished biologist to believe in god, or to attempt the reconciliation of that belief with the findings of modern science. In this book* he touches on many of the common arguments, but makes no excessive claims for them, alone or together. Human societies have commonly adopted religions, however disparate—thus, religious belief may be held to be natural to man. Human moral values have varied, and do vary, but to possess them at all is evidence of divine inspiration. The subjective, but clearly not rare, experience of an exaltation, which does not have an obvious explanation in drugs or other definable agents, is in one sense quite real. Hardy sees them all as simultaneously manifestations of natural law (with their analogues present in infra-human species) and of the divine.

Thus he is prepared to see a dog's adoration of its master as equivalent to man's of his gods. Life in a hunting pack gave selective advantages to the capacity to be a follower. The rituals of worship he finds to be ethologically respectable gestures of submission, and this leads him to invite us not to feel embarrassed at reciting the Lord's prayer on our knees. It is nature's way of expressing devotion. But to what? To nature, seems to be the answer.

Indeed, if I understand Hardy's position correctly, it is not easily distinguishable from pantheism, and this will present both his theist sympathisers and his atheist critics with difficulties. The gods which old-fashioned atheists are accustomed to be without are fairly well defined. Their interventions in our affairs are particularly impressive when they flaunt natural law. As swans or as the holy ghost they impregnate women and tangible offspring are the result. They listen to human prayers and may or may not, as they see fit, save the king, connive at the destruction of Troy, or give us our daily bread. But Hardy's version of natural theology must leave us very much in doubt about what it is we are being invited to accept or provoked to reject.

One difficulty lies in his rather ambivalent approach to "paranormal" phenomena. He discusses extrasensory perception (ESP) telekinesis and apparitions, but rather tentatively. Paradoxically these phenomena are only especially relevant if they do not normally occur. If ESP and psychokinesis are reproducible phenomena they are on the agenda of science. If they are not, but occur as rare and unpredictable events which might reflect the decisions of a supernatural power, then it is open to believers to use them in support of the hypothesis of god. We cannot forget, however, that our history is littered with phenomena which started as unpredictable, but are now regarded without undue awe—floods, for example.

We may accept that otherwise sane and sober people have 'seen' apparitions, without admitting that these were objectively visible. Hardy does not imply, for example, that a stranger bursting in on one of Canon Phillips' interviews with the ghost of C. S. Lewis would have seen it too. The question is whether dreams, apparitions, and the like are going to yield entirely to psychological, neurophysiological and psychopharmacological analysis, or are events through which supernatural powers have access to our minds. Hardy seems to want to have it both ways. He accepts scientific analysis and the natural origin of the human faculties he discusses, and he is therefore forced to find the divine permeating nature, but only achieving its full stature with the evolution of man. For this, I suspect, traditional believers will not thank him. A god that is not in some sense independent of nature cannot really compete with Zeus.

Hardy presents his views discursively, modestly, and for my taste in altogether too good-natured a manner. If atheist biologists, such as Monod, are wrong their works should be criticised, not combed for sympathetic quotation. Just a little asperity would have done this book a world of good.

D. R. Newth

The Biology of God; a Scientist's Study of Man the Religious Animal. By Alister Hardy. Pp. 238. (Jonathan Cape: London, July 1975. £4.50.

Origins of modern science

ROBERTUS FLUDD ALIAS DE FLUCTIBUS OXONIENSIS MEDICINÆ DOCTOR ET ARMIGER &c

Collection Allen G. Debus

Robert Fludd (1574–1637). From the Integrum Morborum Mysterium: sive Medicinae Catholicae Tomi Primi Tractacus Secondus *(Fitzer, Frankfurt, 1631)*

ALTHOUGH some might be misled by the title of this book† into expecting rather more than is delivered, this is nonetheless a very useful and interesting book. What it does not provide is a serious discussion of the relationships between the three 'themes' of the title. But it does contain useful essays on the separate aspects, as well as a review of the current controversy among historians of the scientific revolution.

The most provocative essay, from the 'mystical' side, is that on Newton's alchemy, by Professor Richard Westfall. Having argued in earlier studies that Newton was not above breaking the rules of scientific method, Westfall describes the enormous bulk and complete technical mastery of the Newtonian alchemical corpus. What else can be said? Newton was clearly committed to chemistry far more than his publications indicate: he was sure that the alchemical tradition contained important secrets; and yet it is highly unlikely that he participated in the mystical aspects of the great work. But was it only chemistry? The matter rests undecided as yet.

A reminder of the importance of the genuinely alchemical and mystical traditions even in the 17th century is given by Alan Dobus. He reviews the career of von Helmont, the mildly Paracelsian chemist, whose sufferings at the hands of the Inquisition are totally neglected in the folk histories of science, perhaps because they were in the cause of a 'prescientific' world view. Also, there is Robert Fludd, the propagandist for a mystical philosophy, who was a close friend of William Harvey, and who defended the circulation of the blood against Gascendi's claims of the existence of the interventricular septum.

Another lively topic canvassed here is the quality of Galileo's reports of his astronomical observations. Paul Feyerabend has accused Galileo of faking these and other reports, and certainly the matter is far from straightforwar.d Although the experts (Righini and Gingerich) disagree, there seems a consensus that it was more a matter of a combination of genuine difficulties, haste and wilfulness than a deliberate or serious deception. Other Galilean topics include an identification by A. C. Crombie of the textbooks from which Galileo derived his early Aristotelean ideas; and a scholars' debate, perhaps unresolvable, on the interpretation of manuscripts purporting to record early experiments on free fall.

A general view of the problem of this book's title is offered by Professor Rupert Hall and Paolo Rossi. Theirs are polemical pieces directed against the advocates of the 'influence' of the alchemical–mystical tradition on the new science. Certainly, they could not find a more knowledgeable and sympathetic antagonist than Rossi, since his studies on Bacon were among the first to exhibit the interplay of continuity and change in the origins of the new natural philosophy from its roots in traditional philosophies, rhetoric, the mechanical arts, and magic. Yet he has chosen to refute rather than assimilate the new claims.

The absence of an ecumenical survey of the problem reflects the fact that at root the debate is ideological. With all that we may now have to admit of the imperfections of the enlightenment and the positivist views of science and its origins, to some it is still a question of rationality in eternal struggle with the irrational. I personally think that Professor Hall is too restrictive, as when he condemns Namierite history as 'irrational' because it ascribes private personal motives for political action. But he and Rossi are together in what seems to be their implicit understanding of the 'irrational', as any view that supposes the faculty of 'rationality' to exist outside the minds of man. By such a criterion much of the hard scientific work on which the scientific revolution was based was accomplished by men who fall wholly or partly under the ban, as Gilbert, Kepler and Harvey. Clearly, the matter needs further analysis.

As a record of a symposium, this is a testimony to the skills of the organisers in bringing together an unusually distinguished and lively gathering. As an exploration of a new facet of a perennial problem, 'the origins of modern science' it represents a good start.

J. R. Ravetz,

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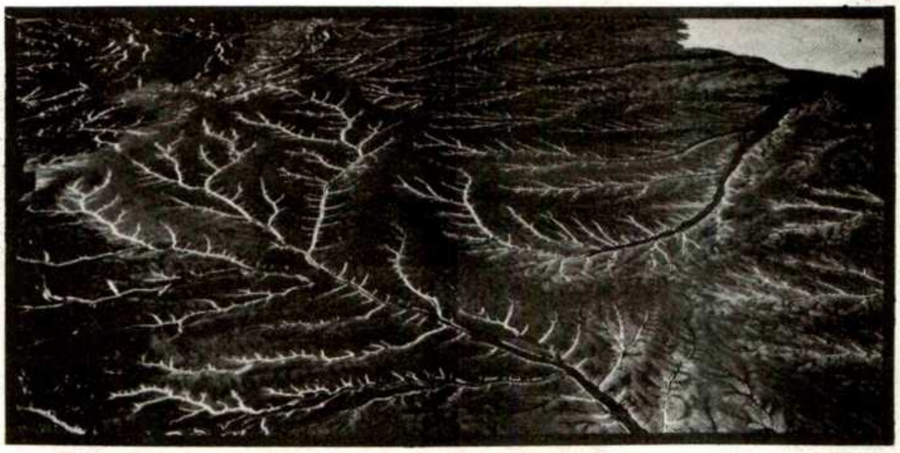
†*Reason, Experiment and Mysticism in the Scientific Revolution*. By M. L. Righini Bonelli and William R. Shea. Pp. 320. (Macmillan: London and Basingstoke; Science History: New York, 1975.) £13.00; $20.00.

Fluvial controversy



Border Island, Arctic Canada

The Work of the River: A Critical study of the central aspects of Geomorphogeny. By C. H. Crickmay. Pp. xvi+271. (Macmillan: London and Basingstoke, January 1975.) £12.00 net.

For over fifty years the author of this book has been concerned with the study of physical geology in the field and the interpretation of scenery in terms of fluvial processes. Here he summarises his findings and theories in a format suitable for students and indeed for anyone interested in river-shaped landscapes.

First, the reader is introduced in an elementary way to the forms of moving water on land surfaces, to the nature of the seasonal work of rivers and to the physical attributes of riverine environments. There follow more advanced discussions on fluvial dynamics and channel equilibrium relationships, with much on grade and interesting comments on other aspects such as cavitation. The erosive work of rivers is then outlined with special attention to base level and a chapter is devoted to examining the hypotheses that constitute the beliefs of several schools of geomorphology.

How then does the author interpret real scenery or landforms seen in the field today? To him the key lies in the great difference between the rate of erosion in river valleys and on flat areas. His main postulations are the hypothesis of unequal activity and the concepts of activeness and stagnancy in landford development. "Some areas of the Earth's surface are almost immune from the postulated [by others] universal destruction by wasting". Ground that is nearly flat or gently sloping (that is, with slopes of under about 5°) makes up the main part of the land surface of the Earth. This abundance of flat land in patches of all sizes up to vast expanses "demonstrates that most denudation is horizontally pursued" by the lateral swinging of rivers mainly at grade. Natural scenery consists of an endlessly varied combination of flats and slopes but since absolute horizontality is rare the Earth's surface may be classified into very gentle gradients or flats and steeper gradients or slopes. Their most significant general relationship is the repetition of one above the other vertically. The flats are stagnant, the slopes active. If stagnant, a geomorphic form may persist almost indefinitely. Although rivers are active erosion agents and wasting may break down all steep slopes, there are certain tracts that survive this destruction because they are out of its reach. When the peripheral flatness extends into the central remnants and predominates there, the land has become a true *Endrumpf*. The ultimate product can only be one broad, low level flat.

These general assumptions mean that most existing scenery was made in time long past and is today essentially relic material. Mountains are areas of "activeness", and the author takes the view that "periods of deep diastrophic calm" are longer than epochs, such as the present, which are characterised by diastrophic agitation and frequent uplifts. The inclusion in the arguments of what to many readers will seem the obvious and the controversial is characteristic of the whole theme. But much of the accepted creed is clearly stated and will be of considerable practical value to earth scientists. Controversy seems inevitable in these natural sciences. For example, is continental drift or mountain building ever still? What is the base level of erosion when low tide mark is far below sea level? Would not high tide and waves consume any flat land mass at base level? But this is the sort of controversy the author deliberately sets out to provoke. Students and earth scientists will find his book a stimulating addition to their bibliographies and as beneficial as a dose of vitamin C after a long season of hibernation.

Robert P. Beckinsale

Evolution of algal ultrastructure

THE green algae are now generally accepted as the evolutionary progenitors of land plants and this book* is an attempt to discuss evolutionary trends within green algae on the basis of cell ultrastructure. After a general introduction, six chapters are devoted to a detailed examination of the ultrastructure, cell division and reproduction of an order of the Chlorophyta. Within each order a restricted number of genera are used to illustrate the diversity of cell structure and reproduction. This approach, although suffering from the potential hazard that the genera selected may not be representative of their order, has been necessary because of the lack of sufficient ultrastructure studies of many green algae. The description of each genus is accompanied by numerous light photomicrographs and electron micrographs (both transmission and scanning), many previously unpublished, which are of consistent good quality and well reproduced. The profusion of photographs accompanying some genera has unfortunately resulted in a loss of synchrony between the text and relevant photomicrographs, such that the reader is obliged to constantly refer to figures several pages distant from the text.

The final chapter is devoted to a discussion of possible evolutionary trends within the green algae by comparison of certain ultrastructural characters. It commences with a list of suppositions concerning primitive and advanced characteristics. The unicellular condition, the possession of basal bodies, flagella, a closed centric spindle, a phycoplast and cell division by furrowing, are deemed to be primitive features, whereas it is suggested that evolutionary advancement can be detected by a colonial tendency, a phragmoplast and cytokinesis by way of cell-plate formation. It is on the basis of the presence or absence of these characters that theoretical evolutionary lines are constructed.

The acceptance of a chlamydomonad cell as the ancestral type of all the green algae is disputed and the hypothesis advanced that this type of cell was the progenitor only of phycoplast-containing green algae. It is argued that the archetypal cell of the other green algae was more like that of *Pedinomonas* (Prasinophyceae). From this ancestral flagellate unicell there developed the filamentous form along two distinct lines. The siphonous line (that is the coenocytic condition) is seen as an evolutionary *cul-de-sac*, whereas evolution by way of ulotrichalean types is suggested as the line leading to the bryophytes and eventually higher land plants.

The arguments for these proposed evolutionary lines are well presented, although occasionally repetitive, and the importance of ultrastructural evidence for evolutionary trends, and also taxonomic classification, is strongly emphasised. The main criticism of this significant work must surely be its price. This has presumably resulted from the inclusion of almost 800 individual photomicrographs, but must place this book well beyond the financial reach not only of students but also of many individual research workers.

T. W. Ford

**Green Algae: Structure, Reproduction and Evolution in Selected Genera.* By Jeremy D. Pickett-Heaps. Pp. vii+606. (Sinauer: Sunderland, Massachussetts; Freeman: Reading, July 1975.) £23.40.

A grimace in an adult female rhesus monkey. Taken from *Primate Behavior: Developments in Field and Laboratory Research*, volume 4, edited by Leonard A. Rosenblum. Pp. viii+407. (Academic: New York and London, July 1975.) $29.50; £14.15.

Evolutionary sex

THIS is an excellent little book† on a big subject. One would think that a topic of such great interest would by now have been adequately understood. On the contrary, within the past few years the evolutionary reasons for the phenomena of sex have become increasingly anomalous.

A few years ago it was suggested that the very existence of sexual reproduction constituted a paradox. For if an organism reproduces by meiosis and recombination with a partner, it 'throws away' half of its genes. An organism that reproduced asexually would have twice the number of its genes represented in the next generation than a sexual one. For sexuality to be selected, an organism would have to possess twice the fitness of asexual ones, and this seems too high.

Presumably, stimulated by this apparent paradox, Williams has undertaken a series of conceptual studies that show how and why this does not really follow. Some of his models are quite convincing, and we are relieved to learn that there are a number of situations in which the fitness of the sexual creatures is high enough to enable sex to evolve. He does not, however, manage to come up with a very satisfactory explanation of why so many sexual organisms exist that do not fit the model. The explanation he proposes is historical accident. He argues that the present-day organisms that should not be sexual are descended from ones that should, and the change for some reason was irreversible. This may be so, but perhaps more adequate hypotheses are in order.

In addition to the big problem, William discusses a number of other issues in the evolution of sex and of hermaphroditism, masculinity and femininity, and some issues in group selection. A number of these matters are given only cursory treatment. For instance, sexual selection is not covered, although sexual dimorphism is reproduction. These include aspects touched on. And the topic of hermaphroditism is made to seem more simple than it really is. I would have appreciated a somewhat longer discussion of these peripheral issues, with more empirical support. Indeed, the lack of facts has been a major drawback in the literature. Although Williams has considered the facts seriously, the reader will perhaps feel a need for more data.

If the facts do not accord with theory, one corrects the theory. Williams's book substantially increases the opportunities for both theoretical and empirical research. Perhaps evolutionary biology is going through a stage like that in astronomy when the epicycles began to become an embarrassment. The time may be ripe for a bolder approach to the subject, and for "theories more pleasing to the mind."

Michael T. Ghiselin

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†*Sex and Evolution.* By George C. Williams. Pp. x+201. (Princeton University Press, 1975.) $13.50.

Pollution of the atmosphere

The Changing Global Environment. Edited by S. Fred Singer. Pp. viii+423. (Reidel: Dordrecht-Holland and Boston, Massachusetts, 1975.)
Cloth Dfl. 95; paper Dfl. 50.
Chemistry of the Atmosphere. By Murray J. McEwan and Leon S. Phillips. Pp. ix+301. (Edward Arnold: London, 1975.) £9.75.

ANYONE curious about the atmosphere and pollution on a global scale will probably enjoy reading these two books. *The Changing Global Environment* is a collection of scientific papers. It gathers together information and ideas of the decade 1963–73 on the possibilities of man-induced adverse changes in the global environment. In the introduction William Kellogg presents a convincing and balanced overview of the possibilities of man-induced climatic change. The authoritative but disparate views of climatology of Murray Mitchell, Reid Bryson and Syukuro Manabe have been expressed elsewhere but they are well worth having side by side in one volume. This is true also of the other sections which concern such problems as ocean pollution and the disturbance of the nitrogen cycle of our planet. Robinson and Robins efficiently summarise and bring up to date their inventory of the sources of atmospheric pollutants and compare them with the outputs of the same compounds from natural sources. The book comforts neither the doomster nor the advocate of unrestrained industrial growth but most of the papers are thoughtful and thought provoking, and will make this collection valuable for a while as a source book for those concerned with global pollution problems.

Just as the environment is changing rapidly so is our knowledge about it and inevitably such a collection has the frozen quality of a group photograph. The participants of the debate all seem to have been caught in mid-sentence. The slightly unreal air of this book is well captured in the second paragraph of the introduction: 'We are now in a period of extensive glaciation. The previous interval occurred 300–250 Myr ago when even the Sahara was glaciated. Of course at that time it was in the position of the South Pole'.

Almost all the authors are American and they occasionally forget that the US is not the world, but this is an inevitable consequence of the real concern in America over global environmental problems and of their generous support of research in the field. By comparison we in Europe tend to take a regional view and a similar collection of European papers would be dominated by the impression that pollution and sulphur dioxide were synonymous. Elsewhere in the world with but few exceptions a narrow parochialism prevails on this topic.

The second book *Chemistry of the Atmosphere* is a lively and enthusiastic textbook of chemical aeronomy. Again the theme is set in the second paragraph of the introduction. 'To a chemist the atmosphere appears as a continuous large scale photochemical experiment. As an experimental system it is unusual in being agreeably free from wall effects . . .' The book is proudly and unashamedly specialist, so much so that the authors are prepared to recognise this limitation to their approach. They say that even the contributions of biologists and poets can be illuminating, but the reader will seek in vain for any information on that part of the air in which the birds fly or where the weather is. Indeed the only mention of the troposphere is in chapter 7 where the chemistry of air polution is considered. This is no criticism of a book which is a splendid introduction for students of chemical aeronomy and for those interested in the scientific basis of important contemporary global problems such as the alleged depletion of the ozone layer by odd nitrogen and odd chlorine.

In this fast-moving field no book can be up to date at the time of its publication. Nevertheless the care taken by the authors to emphasise what was unknown at the time of their writing makes the additions and completions of contemporary knowledge fit comfortably with their text. The authors seem to have that personal familiarity with the practice as well as the theory of their subject which makes its explanation comprehensible and a pleasure to read.

Neither of the books enlightens the current issue of ozone depletion by chlorine-containing compounds such as the fluorochlorocarbon aerosol propellants, neither do they consider the probable future issue of ozone depletion by man-induced increases of natural nitrous oxide emissions from the soil. They should be read while their contents are still relevant.

J. E. Lovelock

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S.J. Pirt B.Sc. Ph.D. 1975. 284 pages, 89 illustrations. £10.50

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Circle No. 16 on Reader Enquiry Form.

Penguins

The Biology of Penguins. (Biology and Environment.) Edited by Bernard Stonehouse. Pp. ix+555. (Macmillan: London and Basingstoke, March 1975.) £18.50.

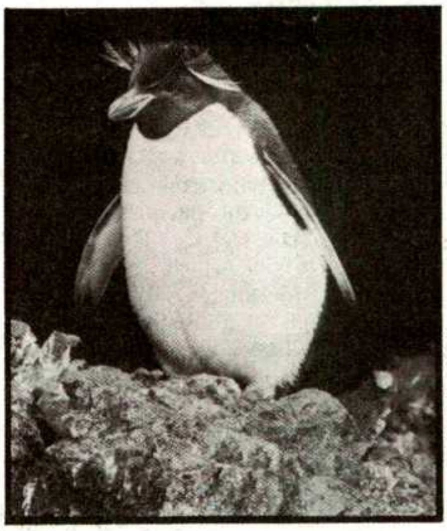
THIS book is the first in a new Macmillan series—Biology and Environment. It is a contributive work, thoughtfully edited by Dr Bernard Stonehouse, one of the world's leading penguin biologists. Dr Stonehouse has assembled twenty papers, a mixture of reviews and the results of new research, which he has grouped into six sections and which together form a very comprehensive reference work on penguin biology. Extensive bibliographies at the end of each chapter enhance the book's academic value.

Dr Stonehouse's introduction includes a concise account of the distribution and major points of biological interest for each of the 18 penguin species. At first, this seems a little unnecessary in a book so obviously specialised, but for the non-penguin functional anatomists, physiologists, ethologists, ecologists (and others) who will also find the work of interest, this check list provides both useful background information and a ready appreciation of the size and diversity of the family. Professor G. G. Simpson begins the section on evolution and taxonomy with an updated review of fossil penguins, the first to be published since his earlier catalogue in 1946. A more recent approach to the elucidation of penguin phylogeny is reviewed in a chapter on the results of protein electrophoresis.

The section on anatomy and physiology contains five papers, ranging from a detailed study of skull anatomy, particularly in relation to feeding, to a valuable review of diving behaviour and physiology. A report on thermoregulation in the little-studied Galapagos penguin is particularly interesting. The lion's share of the book goes to the section containing three chapters on temperate and subpolar penguins amongst which the crested penguins, which have received little attention in the past, are the subject of Dr J. Warham's long contribution, the first published comprehensive account of the genus. Six chapers make up the section on breeding and population studies of Antarctic penguins and although, not surprisingly, the much-studied Adélie penguin is well represented, it is refreshing to find that information on other species has been included. Notable amongst these is an account, prepared by members of the British Antartic Survey, of the little-known Chinstrap penguin. A useful review of penguin predators, both at sea and on land, joins a specialised study of Emperor penguins to form a short section on predation and mortality. Adélie penguins figure again in a final section on communication and display, consisting of two chapters which provide interesting examples of different approaches to the problem of describing and interpreting behaviour.

Most of the criticisms which can be levelled at the book are explicitly felt by the editor himself. He fairly points out the absence of papers on long term population studies, parasitology and conservation, and particularly of studies at sea where, for penguins as for most seabirds, relatively little is known. Too often, contributive works of this kind are merely collections of loosely connected, highly esoteric papers but this is not the case here. Dr Stonehouse has skilfully compiled a volume which, in spite of its omissions, is sufficiently comprehensive to wear its title honestly. The book is sure to find a ready place in many libraries.

David M. Burn



Photograph: John Warham

Endangered species

Breeding Endangered Species in Captivity. Edited by R. D. Martin. Pp. xxv+420. (Academic: London and New York, May 1975.) £12.80; $33.75.

THIS collection of papers marks the beginning of a very much greater realisation by responsible zoos of the role that they should be playing in preventing the total loss of some fascinating and important animal species which are at risk for reasons usually related to man's rapid expansion.

The editor is to be congratulated on bringing together in a readable form, papers which vary in the quality of presentation and of content. Nearly all shed light on the task of maintaining viable breeding groups in captive conditions, especially those that are rare in the wild. A number of authors have drawn attention to the broader issues involved in the development of breeding programmes, and some rightly demand a more pragmatic approach by those controlling animal collections to ensure the maintenance of viable breeding groups on an inter-zoo co-operation basis, thus avoiding the dangers of inbreeding and gene-pool loss. Attention is also drawn to the need to maintain rare animals in a number of collections rather than concentrating them in one area where they may so easily be destroyed by disease or other misfortune. Views are expressed as to whether the genetic changes occuring during, and possibly as a result of, captive breeding will fit animals for a return to a natural environment, especially if re-introduction is attempted after a number of generations in captivity.

Pilot projects to ascertain the problems of re-introduction are suggested, and these could as easily involve common species. In this context, it should be remembered that today's common breed could so easily be tomorrow's rarity, and that every item of information that might help to maintain these in a productive captive state should be available to those involved in this.

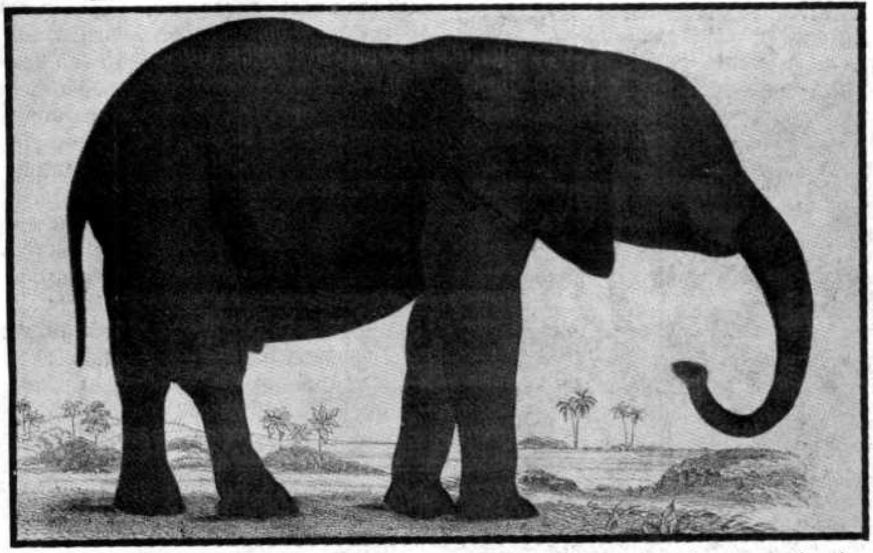
The Fauna Preservation Society, one of the conference sponsors, has been for many years responsible for the conservation of animals in their natural state, and has been perhaps identified with conservationists, many of whom are still unable to see the value of breeding endangered species in Zoological Gardens. The other sponsor, the Jersey Wildlife Preservation Trust, on the other hand has been at the forefront of those zoos who specialised in breeding endangered species; but in this field also there are regrettably still many zoos who do not believe their function is other than to entertain. It is the directors of these zoos that Gerald Durrell would like to see on the endangered species list.

The publication is unfortunately rather highly priced; unfortunate because it should be an essential part of every zoo director's library, and not just those Directors who are already aware of what has to be done. In a wider sense it should be on the bookshelves of all those who are deeply concerned with the future of existing wildlife and wild places.

This collection of papers may be the first of a succession of books on the important challenge of ensuring for moral and other reasons, species of animal life for the aesthetic and other pleasures of future generations. Most first efforts have their imperfections, and this is no exception, but it does form a foundation on which to expand.

R. J. Wheater

Elephants of Uganda



Mary Evans Picture Library

Elephants and their Habitats: the Ecology of elephants in North Bunyoro, Uganda. By R. M. Laws, I. S. C. Parker and R. C. B. Johnstone. Pp. xii+376. (Clarendon: Oxford; Oxford University: London, July 1975.) £15.

THIS is ecology on a grand scale. The stage is the 1,200 square miles enclosed on the west by Lake Albert and on the north and east by the great sweep of the Victoria Nile from Lake Kyoga to Pakwach. Elephant biology dominates the action, but the book encompasses the whole environment, one in which the changing scene is governed by the population dynamics of the elephant and man's relationship with these animals—in competition or conservation.

Before World War II, and for some years after it, information about elephant populations was plentiful but usually wrong. The most experienced game wardens and hunters had totally inadequate ideas about elephant numbers and movements, especially in the tall grass and forest areas such as Bunyoro. Advance has come with the development of the National Parks, and with the expansion of air travel and the use of aerial surveys. Richard Laws, a Cambridge zoologist, was foremost among the pioneers who applied modern ecological methods to populations of large mammals. He came to Africa after working on whales and seals and has since returned to his Antarctic studies, so that one may suppose him to be attracted by the big beasts and the romance that attaches to them. His approach, however, is matter-of-fact and his research is distinguished for its intellectual rigour allied to a grasp of administrative and economic problems. In this book he is assisted by a wildlife manager who was born in East Africa and a forester who has been particularly interested in the Bunyoro district. The result will serve not only as a mine of information but also as a model of what a study of this sort can embrace.

This book is not about all "Elephants and their Habitats". The subtitle perhaps more strictly describes the content, but it is undeniably relevant to all elephant management. I think it is safe to say that there is no other mammal population in which growth, nutrition, habits, reproduction, age structure and behaviour has been so thoroughly studied in relation to climate, flora and fauna and land-use of its habitat. In north Bunyoro, "by mutual agreement between the individuals, departments and companies involved, all the relevant information has been pooled to form the basis of this book".

There are no adventure stories of life on safari here, and this is not a 'coffee table' volume. It is true practical science, an authoritative account, detailed and for practical purposes complete, for the use of students and the guidance of administrators. After reviewing the history of the region and man's use of it, the authors describe its flora and fauna in some detail before embarking on six chapters of elephant biology. There follows a chapter on the changes in habitat associated with past management and a cropping programme involving both elephant and hippo. In the final chapter the authors put forward recommendations based on their analysis of the ecological factors involved—recognising aesthetic and cultural considerations, but regarding economic considerations as "of overriding importance". Many will judge this book indispensable. **John S. Perry**

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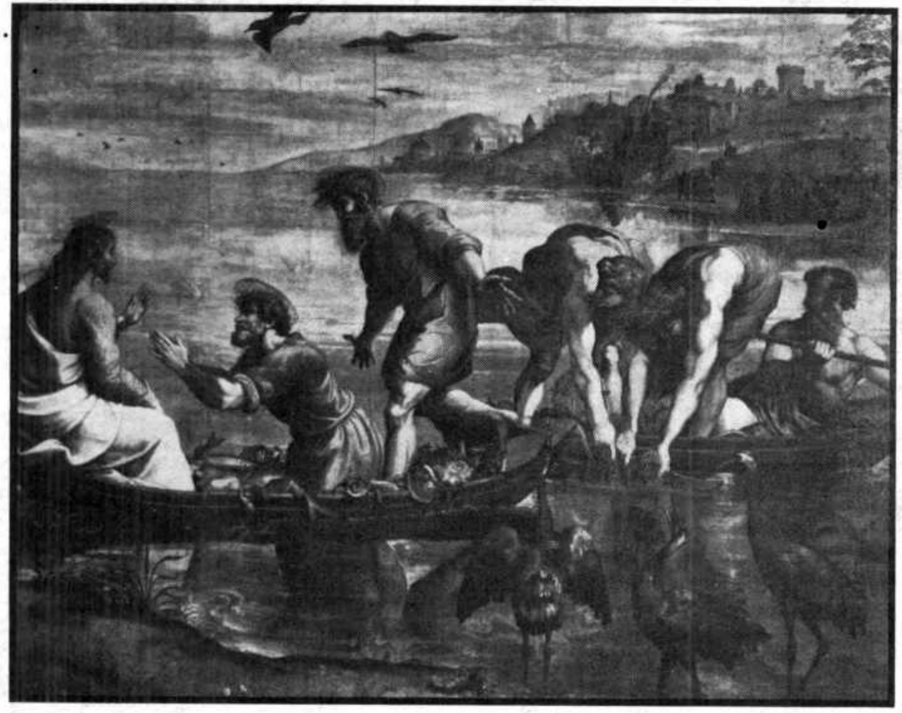
Prospects of the sea



Miraculous Draught of Fishes by Raphael. Courtesy V. & A. Museum

IT can be very salutary to be reminded that nearly 50 years have passed since one became a student, in 1928, the year in which the first edition of "Russell and Yonge" appeared. Well before that I had been introduced to Frederick Warne's well-known (and well-read) *Wayside* and *Woodland* books, but in several respects the appearance of *The Seas* must have marked a high point in the series. This compact book provided, as nothing else had done, for both layman and student, a concise view within one cover of what was then known of the seas and the life they maintain, with a glimpse of the ways in which they were being investigated. For the beginner, and later, the book was as fascinating as it was useful and, as in the whole series, the lavish illustration was a particularly valuable feature. Although it would not be correct to suggest that for this student it was instrumental in deciding on a career (there were other influences) it had its place—and the privilege of being able to listen to the authors at the Bristol British Association meeting in 1930, following their return from the Great Barrier Reef Expedition, also played its part. These two authors must undoubtedly have influenced many in their choice of a career, and aided far more in acquiring a sense of perspective about the life, largely hidden, that inhabits the greater part of the earth's surface.

Very rightly, the book sold well, with a second edition in 1936 (two reprints) and a third in 1963. Now a fourth and extensively revised edition has been produced in a larger format. As the blurb reasonably implies, not many co-authors are fortunate enough (in several ways) to deserve, and secure, such treatment. Not many co-authors, of course, have themselves contributed so much by way of original work (in remarkably complementary ways) to the progress made in marine research over 50 years. Both are still contributing and it is no mere coincidence that each should have been knighted for his service to science.

Their new book is still presented within the old, and very adequate, plan but it is brought up to date in many places. Indeed, collation of the latest edition wtih the old is a fascinating task, and it is a tribute to the care given to the earlier editions that so much can stand today, mainly with supplements rather than major alterations. There are, however, two new chapters—one on "Sensory Perception" and another on the "Influence of Man on Marine Life"—and the earlier chapters on "Shellfish" and "Products from the Sea" have been brought together under the title "Marine Exploitation and Cultivation". So much has taken place, and been discovered in marine research since 1927 that, in a still compact introduction only some of the highlights can be revealed, and interesting discussions might be developed on just what is (and will be) held to be the most significant. For example, there might have been a case for saying a little about the new knowledge gained concerning continental drift, and inferences it may have for life in the seas. Again, although the Torrey Canyon and Santa Barbara episodes are mentioned, there is no reference to taking oil from under the sea in the new chapter on the "Influence of Man", although this is already influencing marine life, and will do so more and more, as also many of the men who earn their living by exploiting the seas in other ways. But where should one stop in what is necessarily an introduction to a vast subject?

This edition also has a largely new set of illustrations, for many of which the authors are indebted to Peter Stebbing, although several of the "classic" plates have been retained, together with a number of photographs by D. P. Wilson. The earlier frontispiece, however, showing a moderately equable sea has been replaced by a striking dust-cover enetitled *Rough Sea!*

This edition should prove as valuable and stimulating as ever. It is well produced and readable in both senses of the work. Even at £6.95 *The Seas* is still, in these days, a good buy.

THIS book† is the latest addition to numerous volumes on the nature, life and problems of the sea which have appeared over the past few years. Dr Bellamy, however, has his own approach to the subject. Starting with a general description of the oceans with a very adequate account of the principal factors influencing sea and shore as environments for life, he proceeds with descriptions of the various forms of marine life and so to some account of the oceans as a source of food and other resources with the effect of man on this, largely by way of pollution.

This book is intended for the general reader who will get a very adequate general idea about the oceans from the initial chapters which include some account of the probable origin of life with an account of cell and plant life in the sea, leaving a description of animal life to occupy the following 16 chapters, almost two thirds of the book.

Here Dr Bellamy essays to describe the full range of marine invertebrates to a general reader and it is clearly on his probable degree of success in this venture that any estimate of this book must largely be based. Although aided by numerous black and white and coloured illustrations, one still wonders to what extent he will accomplish his purpose.

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*_The Seas: An Introduction to the study of life in the Sea._ Sir Frederick S. Russell and Sir Maurice Yonge. Pp. x+283+48 plates. Fourth edition, completely revised and extended. (Warne: London and New York, September 1975.) £6.95 net.

†*The Life-Giving Sea.* By David Bellamy. Pp. 320. (Hamish Hamilton: London, September 1975.) £5.75.

The sponges, to which he possibly devotes too much attention, emerge as much less organised than they really are. The coelenterates get more judicious treatment although there is confusion between the stinging coral, *Millepora,* and the very different blue coral, *Heliopora* and the plate opposite page 65 showing an unnamed green coral is mystifying. The only chlorophyll in corals is contained in the brown zooxanthellae. The mushroom coral, *Fungia,* cannot "move about on the bottom"; it can only (if small enough) be carried about by water currents, although it can right itself if turned over and uncover itself when buried under sand.

The author is clearly fascinated by ctenophores (comb jellies), describing not only British species but the glorious Mediterranean Venus' Girdle and even the exotic *Coeloplana* and *Tjalfiella.* But this is unfortunately balanced by a very inadequate treatment of the far commoner and vastly more important Crustacea and Mollusca, his accounts of which one feels would only give a confused idea to any previously uninstructed reader. There is no mention of the ubiquitous amphipods and isopods; moreover crabs, lobsters and shrimps do not have nauplius larvae.

The structure of a bivalve mollusc is only indicated by an inadequate diagram and indeed throughout there is little mention of benthic organisms generally (apart from fixed algae) in spite of their fundamental importance in marine productivity. Echinoderms are better treated although the arms of brittle stars are surely not "festooned with tube feet".

There is much better treatment of the fish where, of course, even an unexperienced reader can meet the author part way. The major forms and modes of life are well described in the text and illustrated in the figures. Dr Bellamy continues through marine reptiles and birds to sea cows (a pity he says so little about these, with only an uninformative photograph of a manatee) to the whales, and a very interesting account of the work of Gerald Kooyman on the Weddell seal. As to the Californian (rather than the "southern") sea otter, the story is now changing, with the protected animal devouring every available invertebrate, including worms!

The final chapters cover marine productivity, including coral reefs, with the extent to which this is being affected by man (although whether man's activities are responsible for the current plagues of coral-destroying crown-of-thorns starfish is dubious). But here Dr Bellamy most fittingly emphasises the significance not merely of the evolution of diverse animal forms but of "an integrated living system that mirrors the potential of the environment". One also approves his description of "pollutants" as "enrichers, suspenders, cloggers and killers" and his discussion on the relative importance of these categories.

Criticism could be made of various statements including the strange confusion—at any rate to one of this reviewer's age—of Rider Haggard instead of Conan Doyle as the originator of the *Lost World.* The plumose anemone is repeatedly termed "plumrose" and has *Gonyaulax* now become *"Gonyaulux"*? A final criticism concerns the author's tendency to write down to a popular level, to refer to leeches (beautifully adapted animals) as "nasty, horrible, revolting", to edible Crustacea as "mouth watering", and the use of "mind boggling" elsewhere.

Nevertheless this book has the major virtue of enthusiasm, reflecting so much of what David Bellamy has personally seen and done. He makes admirable use here and there of statistics, and the illustrations, although inevitably varying in quality (the one claiming to represent a fiddler crab surely doing so in error), are generally both apposite and informative. And finally, this is a most pleasant book to read.

C. M. Yonge

Dynamics of fishing

Marine Ecology and Fisheries. By D. H. Cushing. Pp. xiv+278. (Cambridge University: Cambridge, London, New York and Melbourne, July 1975). £9.00; $27.50, hard cover: £3.90 paperback.

FEW attempts have been made in the past to bring together the population dynamics approach to fisheries science with the more fundamental aspects of marine ecology. Fisheries scientists concerned with heavily exploited stocks have, naturally, been preoccupied with stock assessments, fishing mortality and recruitment and have thus been concerned largely with adult and juvenile fish.

Varying recruitment to individual year classes of fish has long been recognised as a factor of major importance. The events which lead to variations in recruitment, however, are of such complexity that they have understandably discouraged efforts to integrate them into models of fish populations. They, in fact, embrace virtually the whole of marine ecology. Nevertheless, if we are to reach any real understanding leading to an ability to predict, they cannot be ignored.

Dr Cushing's book embraces material he used in a series of lectures at Oregon State University and I, personally, am sorry I did not hear the lectures. His competence in presenting the subject in written form makes it readily assimilable, and the presentation is a pleasing example of the competence of Cambridge University Press.

The first four chapters are devoted to a useful condensation and appraisal of information, widely scattered in the literature, concerning the process of organic production in the sea. Space and time variations in the broad ocean areas are dealt with first, then the production cycles in the highly productive but unstable upwelling and divergence regions. The role of nutrients is then considered and, finally, the various attempts which have been made to model organic production in the sea are reviewed.

The next three chapters assemble information about fish stocks and migrations, their growth and mortality, and population dynamics. Again, this is a valuable condensation of a great deal of material widely scattered in literature from all over the globe.

The author then embarks on an exercise integrating our knowledge about the stocks of herrings off northern Europe with the organic production cycles—establishing a convincing correlation. Herring are planktonic feeders but behind the simplicity of this statement, the true complexity of the foodweb which culminates in herring is perplexing. It is illustrated in a critical feeding path diagram and the author explores the approaches which can be adopted to express the relationships in terms which can be handled in models.

Temporal changes and the influence of climatic factors are considered in the two following chapters and the book concludes with a discussion of the interaction between fisheries research and marine ecology, carrying a clear message that they can be mutually beneficial.

Students will find the book extremely useful and marine ecologists and fisheries biologists will broaden their outlook by consulting it.

R. I. Currie

Fruition of flowering plants

Bulbous Crowfoot, Mary Evans Picture Library

The Morphology of Angiosperms: The Structure and Evolution of Flowering Plants. By K. R. Sporne. Pp. 207. (Hutchinson: London, February 1975.) £3.50 cased; £2.00 paper.

THE study and interpretation of plant morphology is a subject which captured the imagination, and occupied much of the research effort, of botanists of the nineteenth century. Yet within the last few generations the emphasis of teaching and research has swung away from the structural and toward the physiological, biochemical and ecological aspects of the subject. Although such emphases are bound to change as new techniques and ideas come to prominence, it is sad that so many of those who teach in our schools and universities have come to regard all matters structural as the descriptive studies of a former era and therefore irrelevant to their needs. Even the word 'descriptive' is held in disrepute in some quarters. Undoubtedly the lack of a good modern synthesis of morphological concepts has contributed to this situation and for this reason Sporne's trilogy of morphological texts is especially to be welcomed.

This final book, dealing with the angiosperms (previous ones have covered pteidophytes and gymnosperms), couples a careful selection of information with a lively and readable style—both necessary attributes of a book which seeks both to enlighten and to interest the student. The arrangement of its contents is traditional, dealing with embryology, then the major structural features of the angiosperms, meristems, root, shoot, pollen, ovules, seeds, and so on. Yet the material of this book is conveyed in a far from traditional manner. There is a strong underlying theme of evolutionary trends, which both provides a unity to the examples quoted and also serves to raise those basic questions of origins which should be central in the minds of all biologists. Sporne's book excells in that he is prepared to raise these questions and attempts to answer them even at the risk of overgeneralisation.

The paucity of fossil records relevant to angiosperm phylogeny means that comparative morphology is still the most useful approach to the questions of evolution within the group. Sporne describes the modern numerical work in this field and, although he recognises its weaknesses, can unashamedly state that "a policy of caution would take half of the fun out of research". Perhaps it is the overcautious approach of his predecessors which has taken much of the fun out of plant morphology, but this lively, informative and entertaining book should succeed in injecting some renewed vigour into this neglected subject. It will be weclomed, and should be read by all students and teachers in the field of plant biology.

P. D. Moore

Science as an obstacle course

Problems of Scientific Revolution: Progress and Obstacles to Progress in the Sciences. (The Herbert Spencer Lectures 1973.) Edited by Rom Harré. Pp. vi+104. (Clarendon: Oxford; Oxford University: London, July 1975.) Paper £1.75 net; Boards £4.00 net.

I MUST be honest. I have never actually managed to get around to reading much philosophy of science, except for Medawar in passing. My reactions to this book are therefore probably akin to those students who listened to the 1973 Herbert Spencer lectures recorded therein. I hope it is not through naivety that I found most of the lectures coherent and stimulating.

The book has six contributors ranging from practising scientists to professional philosophers. The mixture works well, allowing a wide-angle approach to the subject. As I cannot believe that it is a *sine qua non* of thinking scientists that they subscribe to the views of Sir Karl Popper, however, it is perhaps surprising that four of the six writers are self-confessed Popperians, another one is guru Popper himself, and only B. F. Skinner does not pay explicit homage.

The progress of science is viewed at its most practical level by W. F. Bodmer. He exemplifies the external factors, especially ethical and eugenic, that modulate the progress of genetics and concludes with rather hackneyed advice to scientists on how to assume a socially responsible position. Sir Hermann Bondi is more concerned with the internal forces that operate. He reveals, again by example, the kind of fits and starts involved in any scientific advance and correctly emphasises the reliance of progress on new technology.

The development of evolutionary theory is Jacques Monod's theme. He shows how the coming of molecular biology has not only added unpredictable layers of flesh to the bones of Huxley's synthetic theory but has at the same time converted it into a theory fully liable to Popperian falsification. He also attacks his critics who cannot come to terms with the advent of man by chance, and not by necessity. B. F. Skinner is even more critical of those who cannot overcome the obstacles he sees to the progress of behavioural science. Their fault, he argues with clarity, is in being trapped by an introspective, reductionist approach. Unfortunately he fails to develop his own alternative approach and its controversial application to, for example, behavioural modification.

The least tangible of the contributions is that in which J. R. Ravetz suggests that the way to salvation for the present condition of science lies in the spirit of Francis Bacon. The longest essay, replete with footnotes, is Popper's. It is divided into two parts. The first is concerned with his fallibilist theory but with several unexpected diversions. The second part deals with hindrances to scientific progress—economic factors ("big science may destroy great science"), intolerance plus dogmatism and, most pernicious, the danger of allowing scientific revolutions to become blurred by ideological entrenchment or the fashion-mongering of intellectuals.

This book is ideal for undergraduates or postgraduates. By and large it avoids being doctrinaire and is sufficiently down to earth to have a lasting and beneficial influence on its readers.

Peter Newmark

Science Between Culture and Counter-Culture. By C. I. Dessaur, Aren Naess, Everett Reimer, Hans J. Eysenck and A. G. M. van Melsen. Pp. 108. (Dekker and van de Vegt: Nijmegen, 1975.) Dfl. 19.50.

THE debate that has developed in

recent years between the proponents of culture and counter-culture has often tended to focus on science as the chief target for criticism from all sides. This issue was at the centre of the public exchange of views organised in 1974 for the tenth Lustrum of the Catholic University of Nijmegen, in the Netherlands. Five of the papers presented on this occasion have now been published: a general introduction by Professor Desaur (Nijmegen); an indictment of science on 13 different counts by Dr Arne Naess (Oslo); an autopsy of the education system—"the school is dead"—in the context of world wide transformation of culture by Dr Everett Reimer (Puerto-Rico); a defence of science against indiscriminate rejection which often chooses to ignore uncomfortable facts rather than learn to live with them, by Professor Eysenck (London); a reflection on the place of the Catholic university between culture and counter-culture by Professor Van Melsen (Nijmegen).

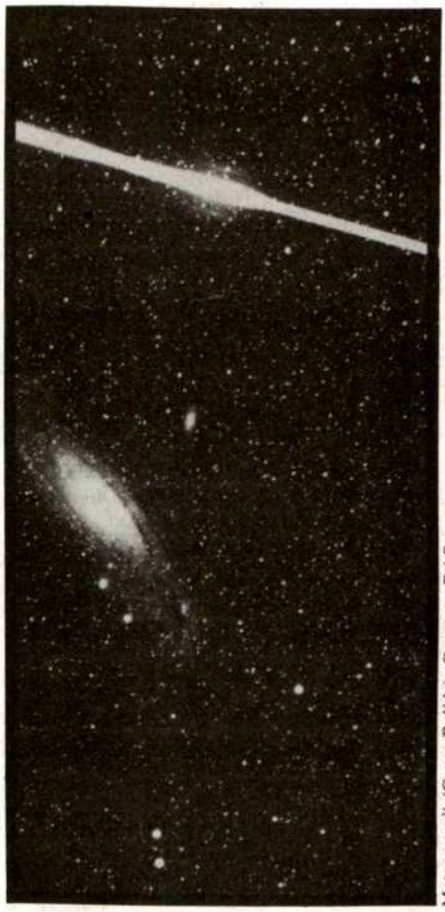
Critics of an enterprise such as science which exerts enormous influence on all aspects of society can always find something on to which they can latch. Defenders, on the other hand, can be quick to point out the undeniable benefits arising from scientific research; as a last resort, they can even denounce technology as a monster bred by the power structure—but for which scientists themselves will not claim responsibility, their concern being the quest for knowledge, and the quest for knowledge only.

When conducted on this level—which is more or less that of the papers presented here—the debate on the pros and cons of science is never-ending and inconclusive. It is true that each society has the science it deserves, and not surprising that "science has become the whipping-boy" (Eysenck) of those who are most critical of a society which has founded wealth and might on the exploitation of knowledge. Certainly, not all scientists have been indiscriminate and uncritical servants of that State, but most have.

The debate between culture and counter-culture seems at times merely to reformulate some of the fundamental issues discussed by C. P. Snow many years ago: why do we have two cultures—stemming from the natural sciences on the one hand and from the humanities and social sciences on the other—apparently incapable of nourishing each other? Why is it that the scientific establishment sometimes presents the appearance of a new theocracy? Why has the scientific enterprise tended to become an element of consolidation of the *status quo*, rather than an instrument of change? • These questions remain unanswered.

Georges Ferné

Source of meteorite material



Meteor trail (Great Bolide), Courtesy RAS

Carbonaceous Meteorites. (Developments in Solar System and Space Science, vol. 1.) By Bartholomew Nagy. Pp. xiv+747. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl. 210; $30.95.

RETURNED lunar material apart, carbonaceous meterorites have received more intensive study per available unit mass than any other natural assemblage of minerals. The unique characteristics of carbonaceous meteorites marked them out from all other types of meteorite as soon as detailed studies began in the 19th century. The French chemist, Berzelius, examined the Alais fall—the first carbonaceous meteorite to be recognised—and reported: "These stones are different from all other meteorites because they look like solidified clay and because when they are placed in water they disintegrate and give off a clay-like odour."

Dr Nagy includes Berzelius' report, along with several other early accounts of carbonaceous meteorites (one unfortunately dated 1984), in his lengthy and detailed survey of these objects. The report continues: "The question arose in my mind, does this carbonaceous earth contain humus or a trace of other organic substances? Could this give a hint to the presence of organic formations on other planets?" We have here, at the beginning of work on carbonaceous meteorites, a statement of one of the major reasons for the concentration of attention on them during recent years. They pose the question of whether any of the carbon compounds they contain can be considered biogenic in origin.

The debate over the answer has revolved round two distinct points: the origin of their complex organic molecules and the nature of the so-called 'organised elements'. Dr Nagy, who has been deeply involved in the investigation of both aspects, devotes over half of his book to this debate. He explains clearly the problems entailed in deciding about origin from physicochemical analysis alone. As he remarks in passing: "Were it not for the fossil remnants in coal, one would most likely still argue its origin." Above all, there is the problem of contamination. Since the compounds of interest are present only in trace amounts, minor contamination can vitiate the results obtained. "A few milligrams of dust which have fallen on and become mixed with 1 g of meteorite, would significantly alter the original hydrocarbon distribution and introduce contaminations which could be mistaken for indigenous compounds." It is hardly surprising that, although general opinion favours an abiogenic origin for the carbon compounds, definite proof is difficult to find.

The agreement that the 'organised elements' do not reflect indigenous life forms is more firmly based. But as Dr Nagy points out, the danger of judging from morphology alone is that judgement may be affected by context. He recounts: "the author once collected unicellular, blue-green algae, killed them in an organic liquid, made a microscopic preparation, and showed it to an experienced mineralogist whose speciality was meteorites. This scientist identified the dead algae as mineral grains from the Orgueil meteorite."

The second important reason for the attention paid to carbonaceous meteorites stems from the belief that they may contain some of the earliest solid material in the Solar System. The chemical, mineralogical and petrological studies that have been encouraged by this belief are described in detail by Dr Nagy, and take up about a third of the book. In this section, perhaps, the author's approach—"It is *not* the purpose of this book to evaluate the validity of reported findings and theories; the reader will have to do this to his own satisfaction"—works less well. The interpretation of the data is so uncertain that rather more guidance would have been valuable. In all, however, this book provides an eminently useful account of the present state of knowledge of carbonaceous meteorites, and can be recommended as a reference source.

A. J. Meadows

Jurassic environments

Jurassic Environment. (Cambridge Earth Sciences Series.) By A. Hallam. Pp. ix+269. (Cambridge University: Cambridge and London, August 1975.) £11.00.

THE Jurassic System has a special place in geological history as it provided the touchstone for early concepts in stratigraphy and biostratigraphy. Just before he died in 1956, W. J. Arkell, in his *Jurassic Geology of the World*, reviewed the global status of these studies in the final of several of his works which are already, in the best sense, classics of geological literature. The subsequent two decades have been revolutionary ones for the geological sciences, with quite new approaches possible through palaeomagnetism and the concept of seafloor spreading: for the Jurassic, too, substantial work has been done in formerly little known areas. The time may be ripe for a dynamic synthesis on the geography, biogeography and ecology of the period, collating and the interpreting of the changing Jurassic environments.

Over the past two decades Dr Hallam has enlivened Jurassic specialists with a range of novel contributions on various aspects of Jurassic environments, contributions which often emphasised the inadequacies of currently held hypotheses rather more than they provided acceptable explanations of their underlying problems. The stimulating role of these in Jurassic studies, especially to a younger generation, has been considerable. It is therefore particularly welcome to have a summary which brings together his views on the sediments, tectonism, sealevel movements, climate, and biogeography of the period.

The most satisfactory parts of the book are where he attempts a rigorous analysis of Jurassic tectonism, sealevel changes and ammonite provincialism, even if length does not permit an exhaustive treatment. These are marked advances over anything which could have been written twenty years ago. Less satisfactory are the instances, especially in the several chapters on various sediment types, which raise problems common to most geological periods. Here, quixotic tilting at various hypotheses when space is not available to deal thoroughly with any of them is not helpful.

Nonetheless this is a refreshing and valuable contribution for those with any interest at all in the Mesozoic Era. For Jurassic specialists the attempt at updating Arkels work, and the long bibliography of more recent work, will be welcome. Students (if any can afford to buy it) will appreciate the breadth of view presented in a work which will add some sparkle to their labours.

How vividly a comparison of this work with Arkell's 1956 volume brings out contrasts in the scientific approach: Hallam delighting in controversy and speculation, Arkell cautious and scholarly. The one giving a stimulating attack on problems, the other working most to ensure a sound foundation for advance. The writing of the one often repetitive and with printer's errors, that of the other always of systematic and elegant prose. Science uses both, but what would Joscelyn Arkell have thought on the fulsome dedication of a volume to him which spells his name incorrectly even on the dedication page?

M. R. House

Cosmic rays

The Origin of Cosmic Rays. (Nato Advanced Study Institutes Series, Series C.) Edited by J. L. Osborne and A. W. Wolfendale. Pp. x+466. (Reidel: Dordrecht and Boston, Massachusetts, April 1975.) Dfl. 105; £39.50.

Composition of Cosmic Radiation. (Topics in Astrophysics and Space Physics, Vol. 11. By Krishna M. V. Apparao. Pp. x+86. (Gordon and Breach: London, New York and Paris, March 1975.) £5.40.

SPECULATION about the origin of cosmic rays has gone on ever since the discovery of the radiation itself, but in the early stages that speculation was inevitably somewhat wild and unproductive. A quite demoralising lack of hard facts about physical conditions in possible source regions and ignorance about the processes which could occur there gave to the discussions a metaphysical flavour, a tinge of the glamour of the occult, that they have never wholly lost.

But times have changed. Fermi's suggestion, that the particles are accelerated by collisions with moving gas clouds during their zig-zag motion through the magnetic fields of interstellar space, brought the cosmic ray question firmly into the forefront of contemporary astrophysics, and there it has remained. Yet the debate on origins is not over. The Fermi model has given way to others in which supernova explosions, supernova remnants (pulsars) and explosive events in the nuclei of our own and other galaxies have been suggested as cosmic ray sources. The experimental data on which these rival models will be judged is accumulating steadily. There have been spectacular improvements in our knowledge of the chemical composition of cosmic rays, their energy spectra, and their directional properties. Optical and radio astronomy—and, more recently, X-ray and γ-ray astronomy—have provided much relevant information about the physical conditions in regions where acceleration and propagation of cosmic rays must take place.

The meeting organised in Durham last year by Professor Wolfendale and his colleagues, with the financial support of NATO, was planned to consider the origin of cosmic rays in this broad astrophysical context, and it is the proceedings of that meeting which are now available in book form. That publication has taken place in so short a time (less than twelve months) is something of an achievement in itself, and although at first sight the price (verging on £20) must seem high, the book is in truth a veritable bargain. The contributors are authoritative, they have written with the needs of students in mind and not solely for their fellow experts, and the material is as near up-to-date as one could hope for. The known facts about the particle radiations are summarised by Watson (Leeds), Thambyahpillai (London), Rasmussen (Lyngby), and Meyer (Chicago), whereas the related γ radiation is considered by Pinkau (Munich) and Stecker (NASA). Relevant features of galactic structure are described by Osborne (Durham) and Thielheim (Kiel), and the link between the chemical composition of cosmic rays and nuclear processes within stars is discussed by Reeves (Saclay). The arguments in favour of the three major models for cosmic ray acceleration are put forward by their best-known advocates: Burbidge (San Diego), Pacini (Frascati) and Colgate (New Mexico). With such a galaxy of authors, the book will surely become a *vade mecum* for every postgraduate student concerned with astrophysics, and a valued source of reference for others.

The second book under review, on the chemical composition of the cosmic radiation, is a much slimmer volume. The author is, once again, an authority in his field and writes to interest students as well as specialists. In principle, he succeeds. Unfortunately, he has been ill-served by the leisurely pace of book production. Important developments, duly noted in the Durham proceedings, are missing in Dr Apparao's book, which carries as its most up-to-date reference "1971—in the press". Although the book is still of interest and has its uses, the potential purchaser must reflect that the price per page is substantially greater than for the Durham proceedings, and that the information it gives him is three years behind the times.

H. R. Allan

States of matter

States of Matter. (Prentice-Hall Physics Series.) By David L. Goodstein. Pp. xii+500. (Prentice-Hall: Englewood Cliffs, N.J., March 1975.) £12.50.

THIS book is based on lectures given at Caltech as an advanced year-long course in applied physics. It is an outstanding example of the nutritious pabulum fed to American postgraduate scientists, and indeed constitutes a very meaty dish. The first chapter (about 90 pages), based largely as the author indicates on Landau and Lifshitz, provides a rigorous introduction to thermodynamics and statistical mechanics. The following chapters then deal with the perfect gas (40 pages), solids (70 pages), liquids and interacting gases (90 pages), some special states including superfluidity, superconductivity and magnetism (90 pages) and finally critical phenomena and phase transitions including scaling laws (50 pages). Each chapter concludes with some shrewd comments on bibliographical sources and some well-tried, rather demanding examples.

Throughout the book the main themes are tackled in terms of thermodynamics and statistical mechanics. This unified, integrated treatment has many attractive features. It has the disadvantage, however, that in some cases the approach is somewhat tortuous. Again the mathematical analysis is often heavy going and rather formal. On the other hand the accompanying discussion, which is expressed in a verbal rather than a written style is always lively and communicative and reflects a first-hand mastery of the issues under review. Those who find the analysis too tough will always find the author's comments illuminating: they breathe life into the dry bones of the formal analysis and provide a fresh and often entertaining insight into the physics of the problem under discussion. There are also some agreeable personal touches—for example, on quantised entities ending in -ons, such as phonons, photons, and so on. "Even human populations have quantised units called persons." On classical theories: so called not necessarily to distinguish them from quantum mechanical, "but simply because classical is what we usually call the theory that came just before the one we believe now." On the irreversible tendency for entropy to increase he quotes the famous quatrain of Omar Kayyam (" . . . the moving finger . . . ") with the comment that "there have been many other statements of the Second Law, all of them less elegant."

The chapter on liquids, fascinating as it is, probably provides material least familiar to the student, and the physics seems to be overlaid too heavily with computational tricks for calculating the interaction of atomic clusters. Again the inadequacy of pairwise additivity could have been made more concrete if the author had compared, for example, the lattice energy of solid argon with that deduced from pairwise calculations: and if his fifth chapter had dealt with dielectrics (as well as magnetism) he could have linked the problem of additivity with the dielectric properties of matter in the condensed state. This in turn provides a bridge with the van der Waals' properties of liquids and solids. Finally, the author quotes the opinion of some scientists that plasmas constitute the fourth state of matter: it would do no harm if applied physicists were to be taught instead about the colloidal state. This is not only of great importance, it also offers scope for some very nice physics. But these are personal opinions rather than criticisms.

Dr Goodstein's heroes, to judge from his references and comments, are Landau and Lifshitz, Mott and Jones, G. I. Taylor and Galileo Galilei. This fine sense of scientific discrimination and catholicity of interests characterise the stimulating and challenging book he has written.

David Tabor

Aged Ecuadoreans

Michaela Mendieta Quesada, aged 106

The Centenarians of the Andes. By David Davies. Pp. 128+17 photographs. (Barrie and Jenkins: London, July 1975.) £3.95.

POPULATIONS containing numerous centenarians and supercentenarians have been reputed to exist in the Ecuadorean Andes since 1825 (G. Coggeshall, 1825, *Voyages to various parts of the world*), and have recently been noted by Leaf (*Youth in old age*, 1975). This book is an anecdotal but informative study of the distribution, way of life and documentary authenticity of centenarians in Vilcabamba, who have been fairly extensively studied, at least from the standpoint of documentation by Dr Miguel Salvador for the Ecuadorean Government. Ages of 140 and 150 years have been reported, but equally remarkable is the state of physical preservation of people throughout old age. Dr Davies' book contains no demographic tables, so that the population breakdown cannot be ascertained from it. As in Abkhasia, interest naturally focusses on the authenticity of the high age records—these seem better documented than in Abkhasian Muslims, by reason of the care devoted to Catholic birth and burial registers.

At present the high age population seems to be confined to a small enclave of mountain villages, but there are rumours of similar longevity in other parts of the Andes not yet investigated. In the villages around Vilcabamba longevity seems to affect at least three distinct population types. Possible causes of survival (genetic endowment, hybrid vigour, low calorie or low fat diet, particular dietary constituents, environment, and absence of psychosocial pressure to age) are discussed, but there is as yet no evidence to support any one of these factors, except perhaps isolation from viruses and continued acceptance of 'old' activity, which clearly operates in the field of work and of sexuality. The environment but not the diet resembles that of Abkhasia, where longevity is reportedly geographic rather than specifically ethnic, affecting Russians, Abkhases and Jews who live in the area.

Dr Davies' treatment is of necessity that of a travelogue, illustrated with some good photographs. A proper monograph giving the demographic picture and the true prevalence of centenarism is overdue, and it is to be hoped, in view of the great interest of the model, that Dr Salvador and the Ecuadorean Government will provide us with one to amplify a previously circulated report (M. Salvador, *et al.*, 1972. *Vilcabamba, tierra de longevos*, Quito: Casa de la Cultura Ecuatorian).

Alex Comfort

Aspects of oceanography

Introduction to Marine Geology and Geomorphology. By Cuchlaine A. M. King. Pp. 309. (Edward Arnold: London, 1975.) £9.90.

Introduction to Physical and Biological Oceanography. By Cuchlaine A. M. King. Pp. 372 (Edward Arnold: London, June 1975.) Boards £11; Paper £5.50.

OCEANOGRAPHY has progressed in the last decade to an even greater extent than most sciences. This is reflected in the fact that Professor King when preparing a second edition of his well-known textbook *Oceanography for Geographers* found it necessary to divide it into two volumes. The first of these deals with marine geology and geomorphology. The book commences with an introductory chapter, which skimpily covers topics as various as deep-sea sediment sampling, diving, station fixing and ocean resources. It continues with a chapter on the structure of the ocean basins in which rather restricted coverage is given to ocean spreading and plate tectonics. The next two chapters, which deal with the continental margins and the morphology of the open sea are very good and probably the most useful in the book. The fifth chapter deals with deep-sea and near-shore sediments and their rates and mechanisms of formation; there is, regrettably, little on their chemistry.

The book closes with a chapter on the origin of ocean water and changes in sea level; the treatment of the former is extremely perfunctory, and no mention is made of the origin of the dissolved solids which give seawater its special character. In general, although some effort has been made to include recent work, this has been done in a rather unselective way. It is particularly disappointing to find no discussion of the results obtained during the Deep-Sea Drilling Program as this has done so much to revolutionise our ideas on marine geology and sedimentology. Although this book can be recommended for students of geography requiring an introduction to marine geomorphology, it is likely to be of much less value to those seeking background reading in marine geology, because of its superficial treatment of the subject.

The second book is an uneasy union between physical and biological oceanography It commences with an introductory chapter which does not relate well with the following chapters and the purpose of which is obscure, particularly when related to chapter 8. Then follow chapters on physical oceanography dealing with the waters of the ocean and their circulation, tides and waves. Much of it is dealt with in a purely descriptive fashion. On the occasions, particularly in chapter 3, when explanations of physical phenomena are offered they are often garbled to such an extent as to suggest a basic lack of understanding on the part of the author (for example, the first complete paragraph on page 141 and the first three paragraphs on page 68 on westward intensification). There are also a number of unjustifiably bold statements of "fact". Thus, the tsunami wavelength (not a well-defined quantity) is stated to be 900 km on page 8 and 160 km on page 9, but surely not in Loch Lomond (page 190). The discussion of the heat budget on page 30, based on work done in 1936, makes no mention of the fact that the individual contributions to it are still not known with any accuracy. On page 33 the impression is given that the only minor elements present in sea water are silver, gold and radium.

The book is out of date in a number of important respects; thus, in chapter 5 the discussion on the observed wind-wave spectrum makes no mention of JONSWAP and the Pierson–Moskowitz spectrum; nor are the currently accepted ideas on longshore and rip currents presented. In many instances important terms are left undefined and even undescribed (for example, PCB, Coriolis force, primary production, and salinity, the latter being only related to chlorinity (itself undefined) by an out of date formula). The text is strewn with minor undefined terms, of which there are six examples in the first paragraph on page 141.

The next two chapters consisting of 94 pages in all, are concerned with biological matters. The first of them deals in a very disjointed way with marine production at all trophic levels. In spite of its key importance primary production is dismissed in about 6 pages. The following chapter deals with the biological exploitation of the ocean, and is primarily a scrappy account of fishing and whaling. The final chapter on "Uses and problems of the oceans", includes subjects as diverse as oceanic pollution, conservation and law of the sea. The author's literary style is an awkward one, and occasionally sentences have to be read several times before they can be understood. Although this book may perhaps be of value for geographers, in the reviewer's opinion it has little to offer to prospective oceanographers or marine biologists, because of its superficiality.

J. P. Riley

Materials and Technology

The publication of Volume 8 in September 1975 sees the completion of this systematic encyclopedia which has been produced for people who want to know about materials, including those who have no advanced training in science or technology. Within each volume each chapter has been written by a specialist or a group of specialists so as to represent a comprehensive account of the subject, which will be of use not only to the general reader but also to those who work with materials in their professional capacity. It will also fulfill a useful function in education as a link between formal textbooks and the practical application of academic learning.

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review article

Mammalian plasma membranes

Mark S. Bretscher* & Martin C. Raff†

1975 is the fiftieth anniversary of the proposal, by Gorter and Grendel, that biological membranes are based on a lipid bilayer. Now well-established, this proposal, like the DNA double helix, was a major breakthrough in molecular cell biology. Here we discuss current concepts about the molecular composition, organisation and behaviour of the plasma membranes of mammalian erythrocytes and nucleated cells.

CELL membrane structure and function are intimately linked to each other and to much of biology. The plasma membrane is the most experimentally accessible and, in many respects, the most important of the cell's membranes It maintains the internal milieu of the cell and has a vital role in intercellular communication. In this overview, we discuss the general features of normal mammalian plasma membrane architecture and behaviour that have been gleaned from recent experiments on lipid bilayers; erythrocyte ghosts and nucleated cells

The lipid bilayer

The three classes of lipids in cell membranes, phospholipids (which comprise the majority), neutral lipids (mainly cholesterol), and glycolipids (largely glycosphingolipids), usually constitute almost half the plasma membrane mass. They are all amphipathic, having a hydrophilic (polar) and hydrophobic (non-polar) end. Phospholipid molecules have a polar head group and two hydrophobic hydrocarbon tails of variable lengths and degrees of unsaturation; when placed in an aqueous environment they spontaneously form bilayers, burying their tails and leaving the head groups exposed to water. Low angle X-ray diffraction studies of various membranes show that the bulk of the phospholipids in these membranes are arranged in the form of a bilayer[1]; this provides the structural framework of the membrane[1-3].

An important advance in understanding membrane structure has come from studies of the physical properties of lipid molecules in synthetic bilayers and selected biological membranes. A variety of techniques, including nuclear magnetic and electron spin resonance, have been used to measure the motion of individual lipid molecules or their different parts[2-4]. These show that lipid molecules in artificial bilayers readily exchange places with their neighbours in their own monolayer[5], but very rarely migrate from one monolayer to the other[6], a process called "flip-flop". Thus, lateral diffusion of a lipid is rapid, each molecule exchanging with its neighbour about 10^6 times a second (ref. 7); in contrast, neighbour exchange across the bilayer by flip-flop occurs less than once a fortnight[8,9]. Nor are individual lipid molecules rigid; their hydrocarbon chains flex and the degree of flexion may be greatest near the centre of the bilayer and least adjacent to the polar head group[10]. Thus the lipid component of a membrane is a two-dimensional liquid in which molecules are restricted to their own monolayer.

The fluidity of a bilayer at a given temperature is determined by its composition. An artificial bilayer of a single type of phospholipid (such as dipalmitoyl phosphatidyl choline) has a sharp and characteristic melting point (or phase transition) at which it changes from its liquid state to a rigid crystalline bilayer (or gel phase)[4] This temperature depends on the nature of the phospholipid head group and on the length and degree of saturation of the hydrocarbon side chains; as might be expected, a high melting temperature is found for lipids with long hydrocarbon chains, whereas the introduction of *cis*-double bonds into these chains (which makes them difficult to pack next to each other) leads to a low melting temperature In artificial bilayers containing a mixture of phospholipids there is a progressive phase change over a broad temperature range, indicating that a "phase separation" into clusters of gel and liquid occurs[4,11].

When cholesterol is introduced into a bilayer, the plate-like steroid rings interact with, and partly immobilise, those regions of the hydrocarbon chains closest to the polar head group (leaving the rest of the paraffin chains flexible); this prevents their crystallisation[4]. Thus, in high concentration, cholesterol abolishes temperature-induced phase transitions; it has the dual effect of preventing formation of crystalline gel areas while inhibiting the overall flexing motion of the hydrocarbon chains. In this way, it imparts an "intermediate fluid condition" to the interior of the bilayer[4].

In thinking about plasma membrane lipids, it is often helpful to draw an analogy between them (as two-dimensional solvents) and water (as a three-dimensional solvent). For example, we may wonder why mammalian plasma membranes contain a variety of phospholipids having different head groups, such as phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine In part, this may be because some membrane enzymes need specific phospholipids for normal function[12,13] It is well known that water-soluble enzymes often require a particular ion for activity and, in a similar way, a membrane enzyme may require an adjacent ethanolamine (rather than serine) head group as activator. Since the bilayer is a liquid, an individual phospholipid molecule could activate several different membrane enzymes over a period of time, just as an ion could for soluble enzymes It has been reported that proteins

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which penetrate into the lipid bilayer tend to immobilise a collar of surrounding lipid molecules ("boundary lipid")[11,12,14,15]. This is comparable with the tendency of water molecules to exist in an ordered state around the surface of water-soluble proteins.

In mammalian plasma membranes, phospholipid heterogeneity and the presence of cholesterol and protein thus modulate the fluidity of the bilayer. The biological importance of membrane fluidity is suggested by the fact that yeast, bacteria and various poikilothermic organisms change their membrane lipid composition to maintain a relatively constant fluidity in the face of changes in temperature[16,17].

There is increasing evidence for significant asymmetry between the inner and outer leaflets of the lipid bilayer of human erythrocytes. Glycolipids seem to be confined to the outer half of the bilayer[18], and radiolabelling[19,20] and phospholipase-mediated digestion studies[21] indicate that most of the choline-containing phospholipids (phosphatidyl choline and sphingomyelin) are found in the outer half of the bilayer, while the amino phospholipids (phosphatidyl serine and phosphatidyl ethanolamine) are largely confined to the inner half. Since the former lipids are more saturated than the latter, head group asymmetry implies asymmetry in the hydrocarbon tails as well; and because only phosphatidyl serine carries a net negative charge, whereas the other phospholipids are zwitterionic at physiological *p*H, there is also a charge asymmetry between the monolayers. Although it is not known whether lipid asymmetry exists in other cell membranes, there is increasing evidence that it does. Since phospholipids do not spontaneously flip-flop at an appreciable rate, it seems likely that the asymmetry arises during biosynthesis[20]. Its biological significance is unknown.

The relationship between the lipids in different membranes of a cell is obscure. Although all membranes can deacylate and reacylate their own phospholipids, only the endoplasmic reticulum has the enzymes required for *de novo* phospholipid synthesis[22], which suggests that phospholipids are synthesised and inserted into pre-existing membrane there. Furthermore, specific proteins in the cytosol exchange phospholipids between the different membrane compartments[22], but how the observed differences in lipid composition between organelles within a cell are generated or maintained is unknown. In erythrocytes, where *de novo* lipid synthesis does not occur, there is a slow renewal and remodelling of cellular phospholipids through exchange with plasma lipids[23]. It is not clear to what extent such exchange occurs in other cells, but most cells seem able to incorporate exogenous glycolipids into their surface membranes[24].

There remains a number of unresolved questions about membrane lipids which could have important implications for membrane function. For example, it is often assumed that each lipid monolayer of a membrane is under the same lateral pressure, but this need not be so. It seems quite possible that the external monolayer is under compression and the inner monolayer under tension. Such an arrangement can be visualised if we think of an area of bilayer with more phospholipids on one side than on the other, but in which each monolayer covers the same area. This is possible because lipids are rather elastic to lateral compression Again, it is unclear to what extent there is compositional or conformational (liquid against gel) heterogeneity within each monolayer, and whether such heterogeneity would have biological significance. A change in lipid conformation can lead to a change in protein distribution in reconstituted membranes[11], but it seems doubtful that membrane protein distribution or function is ever normally controlled by changes in lipid conformation.

Finally, the widespread occurrence of many different glycolipids should be mentioned. In general, their biological function is obscure, although some of the gangliosides have been implicated as receptors for some bacterial exotoxins, such as cholera[24,25] and tetanus[25].

Association of membrane proteins with the bilayer

More than half the mass of most plasma membranes is protein. Unlike the extended monolayer of protein on both surfaces of a membrane originally envisaged[3], membrane proteins seem to be built into the lipid bilayer in a highly asymmetrical way. The ease with which these proteins can be removed from the membrane varies over a more or less continuous spectrum—from those which can be eluted by rather gentle procedures (such as lowering the ionic strength) to those which can only be removed by total disruption of the bilayer matrix. The two extreme cases are sometimes referred to as "peripheral" or "extrinsic" and "integral" or "intrinsic" proteins[26]. In a loose sense, this classification may be helpful, provided that it is not mistaken for a molecular description of how these proteins are arranged in their membranes, which, in almost all cases, is unknown. Some proteins extend across the bilayer; these are believed to have hydrophobic surfaces which associate with lipid molecules by hydrophobic interactions in the bilayer interior. To solubilise them, detergents, organic solvents or chaotropic agents are needed which break hydrophobic bonds and destroy the bilayer; even then, some strongly associated lipid may remain. Detergents, like membrane lipids, are amphipathic; they can be anionic (as with sodium dodecyl sulphate (SDS), deoxycholate (DOC), or Sarcosyl), catonic (such as Cetavlon) or non-ionic (as with the Triton, Brij and Tween detergents, Lubrol PX, Nonidet P40). Their hydrophobic ends bind to the hydrophobic regions of the proteins, largely replacing the phospholipid, and thus bring the proteins into solution as detergent–protein complexes[27]. In some cases, the detergent (such as SDS) also binds to hydrophilic regions of the protein, causing them to unfold. Even with non-denaturing detergents, if the detergent (and remaining lipid) is removed, membrane proteins are often highly insoluble and aggregate in neutral aqueous buffers.

Membrane biochemistry has been revolutionised by the use of electrophoresis in SDS polyacrylamide gels; to separate and study solubilised membrane proteins. In this procedure, the amount of anionic SDS bound is approximately proportional to the mass of the polypeptide. The charge of the detergent overwhelms the intrinsic charge of the protein, which thus migrates according to its molecular weight when sieved by the polyacrylamide; it can later be visualised by various protein stains. Although glycoproteins can also be resolved by this technique, estimating their molecular weights from their electrophoretic mobility is misleading if they contain large amounts of carbohydrate, because the amount of SDS bound per unit mass of glycoprotein, and the hydrodynamic behaviour of the detergent–protein complex, are altered[20,28].

Membrane proteins of erythrocytes

More is known about the plasma membrane of human erythrocytes than about that of any other mammalian cell, mainly because it is the only membrane in these cells and can be readily isolated intact, free from contamination by other cells or organelles. Erythrocyte membranes, or "ghosts", are prepared by hypotonic lysis of red cells, which releases the haemoglobin. By studying intact cells, permeable (unsealed) ghosts, or inside-out resealed ghosts[29], one can study both the external and cytoplasmic faces of the membrane.

When human erythrocyte membrane proteins are studied by SDS polyacrylamide gel electrophoresis, approximately 15 major protein bands are seen, varying in molecular weight from 15,000 to 250,000. The properties of these proteins have been frequently reviewed[20,29–31], so they will not be discussed in detail here. Several important general points have, however, emerged from studies using labelling reagents or proteolytic enzymes to detect which proteins are accessible on one or other (or both) membrane surface(s). (1) All of the proteins and glycoproteins are asymmetrically oriented across the bilayer, being exposed on one or both sides of the membrane. (2) Most of the protein molecules are associated with the cytoplasmic side of the membrane, and of these, most can be removed from the membrane in very low ionic strength solutions. The most abundant protein located on the cytoplasmic side is "Tektin A" (ref. 32) or "spectrin", a fibrous protein which comprises about 25% of the membrane protein mass and which is composed of two separable polypeptides (about 220,000 and 240,000 daltons each). (3) There are two major proteins exposed on the external surface; both are glycoproteins which extend to the cytoplasmic surface and thus span the membrane No protein is found associated only with the external surface of the cell. The larger of the two proteins which span the bilayer (electrophoretic band 3) (ref. 29) is a globular protein of about 100,000 daltons; it has relatively little carbohydrate (but may be a receptor for concanavalin A) (ref 33), and may be present in the membrane as a dimer. This protein is believed to be the red cell anion and glucose channel[20,34,35]. The other (MN glycoprotein=major sialoglycoprotein=glycophorin) is the most completely characterised mammalian plasma membrane protein; it is 131 amino acids long and has a molecular weight of approximately 30,000 (refs 31 and 36). Its amino-terminal hydrophilic end at the cell surface carries all of the carbohydrate (about 100 sugar residues), accounting for the great majority of the cell surface carbohydrate, including more than 90% of the sialic acid, and thus most of the negative charge of the cell surface. Its hydrophilic C-terminal tail is exposed on the cytoplasmic side, while an α-helical hydrophobic segment of 23 amino acid residues spans the non-polar bilayer. This glycoprotein carries some blood group alloantigens (MN, possibly ABO) and acts as a receptor for influenza virus and some plant lectins (PHA, wheat germ agglutinin)[31]. Besides these major proteins, a number of membrane-bound enzymes are present in such small amounts that they do not show up as distinct bands on gels; nonetheless, it seems from functional studies that some of these also are situated asymmetrically across the bilayer[29].

At present, freeze fracture electron microscopy is the only way of visualising the interior of a cell membrane. In this process cells are fractured while frozen in a small block of ice. The fracture plane passes through the middle of the bilayer[37], exposing two complementary fracture faces, which are shadowed with platinum and carbon. The resulting replicas, examined in an electron microscope, reveal that the inner (A) and outer (B) halves of the erythrocyte bilayer are studded with small (80 Å) intramembrane particles. These are homogeneous in size and randomly distributed, being more dense on the inner (A) face. Since lipid bilayers and vesicles are devoid of particles unless proteins are included, the particles probably consist mainly of protein. It is likely that to be visualised as a particle, a polypeptide, either by itself, or as part of a subunit aggregate, must span the membrane and be of sufficient size to "cast a shadow" Although it is unproven which proteins constitute the particles in human erythrocytes, much of the evidence is consistent with contributions both from band 3 and from the MN glycoprotein. Thus, when erythrocyte ghost particles are induced to aggregate into clusters (see below), cell surface ABO antigens[38], receptors for the lectins, PHA[39] and concanavalin A[40], and for influenza virus[39] are aggregated in the same pattern as the particles. This suggests that the particles extend to the outer surface of the ghost membrane, and that most are constituted by the two major surface-exposed proteins that are thought to carry these antigens and receptors.

The principal surface-exposed proteins of erythrocytes seem to be relatively immobile[41], and in this respect differ from those of nucleated mammalian cells, in which many of the plasma membrane proteins are able to move about in the plane of the membrane. There is increasing evidence that spectrin forms a filamentous network on the cytoplasmic surface of the erythrocyte membrane and it is this that directly or indirectly holds the particles, and thus the major penetrating proteins that probably constitute them, in place[42]. Thus, intramembrane particles can be induced to aggregate in ghosts in a variety of conditions, but only in ghosts which have been depleted of spectrin; particle aggregation does not readily occur in intact erythrocytes, or in ghosts having a full complement of spectrin[42]. It is not known whether the various minor erythrocyte membrane proteins, such as Na^+–K^+ ATPase or acetylcholinesterase, are capable of translational mobility, or whether they, too, are immobile.

Membrane proteins of nucleated cells

The total membrane mass in nucleated cells includes nuclear, endoplasmic reticulum, Golgi, mitochondrial and various vesicle membranes. The plasma membrane can therefore be studied only after its isolation from these other membranes, or by using membrane-impermeable reagents to label surface proteins. Moreover, reliable methods for making the equivalent of permeable or inside-out ghosts to study the cytoplasmic side of these membranes are still lacking, although isolated phagosomes may well prove useful in this respect[43]. Thus, while it seems likely that a larger variety of membrane proteins and glycoproteins will be found in most nucleated cells than in erythrocytes, little is known about their topographical arrangement in the membrane One very large glycoprotein of cultured fibroblasts has, however, attracted considerable attention[44]. It seems to have a molecular weight of about 250,000 and is accessible to the cell exterior, since it is extremely labile when intact cells are treated with low levels of trypsin. This protein is also sensitive to proteolytic attack from the cytoplasmic side of the membrane (in phagosomes, which contain an inverted region of plasma membrane) and thus probably spans the fibroblast plasma membrane[43]. The great interest in this molecule resides in the observation[44] that it is absent from the plasma membrane of a variety of virally-transformed fibroblasts, which suggests that it may have a role in the control of cell growth.

Perhaps the most important recent discovery about the plasma membrane of nucleated cells is that many of its proteins are mobile, being able to diffuse laterally in the plane of the membrane, and, by analogy with rhodopsin molecules in retinal rod disk membranes[45], to rotate about an axis perpendicular to the membrane plane. The first suggestion of this came from X-ray diffraction studies on rod disk membranes, which indicated that rhodopsin molecules, the major proteins in these membranes, were mobile and in a "liquid" state[46]. The dramatic demonstration, in 1970, that different antigens on the surfaces of nucleated mouse cells and human cells rapidly intermix when the cells are fused by inactivated Sendai virus was the first indication that proteins in the plasma membrane had considerable mobility[47] The following year, it was demonstrated that divalent antibodies, binding to immunoglobulin receptors, or to other antigens on the surface of lymphocytes, lead to the selective redistribution of the antibody–antigen complexes in the plane of the membrane[48]. Three distinct types of redistribution occur. (1) Patch formation, which is the passive clustering of cross-linked macromolecules into aggregates, a process analogous to a precipitation or agglutination reaction occurring in the two dimensions of the fluid membrane[49]. (2) Cap formation, the polar segregation of the cross-linked molecules from other membrane components by an active process, probably dependent on microfilaments[48,50]. (3) Pinocytosis of the complexes, which in some cases may lead to extensive loss of the membrane protein[48,50], a phenomenon referred to as "antigenic modulation"[51] In some systems, shedding of the surface antigen–antibody complexes may contribute to modulation. Moreover, monovalent Fab fragments of antibodies can, in some situations, induce pinocytosis[49] and/or modulation[51]. Hormone-induced disappearance of cell surface hormone receptors, referred to as "down regulation"[52], presumably represents another example of ligand-induced modulation. In each case, the process is reversible: the cells resynthesising the modulated protein when incubated in the absence of ligand. It is likely that modulation represents a normal homeostatic mechanism whereby the concentration of signalling ligand regulates the concentration of its receptors on a target surface.

Patching and pinocytosis can be induced in all nucleated mammalian cells so far studied, if an appropriate cross-linking agent is used, while capping has been demonstrated in many (but not all) cell types, including lymphocytes, granulocytes, and fibroblasts. Besides providing direct evidence for membrane protein mobility, ligand-induced redistribution is a powerful tool for mapping cell membranes. For example, it is possible to determine the relationship between two molecules, A and B, on the surface of a cell by redistributing A with anti-A antibody and seeing what happens to B, if B redistributes with A, the two molecules are associated in the membrane (or become associated after ligand binding), whereas, if B is left behind, they are independent. The two antibodies are followed by conjugating them with different markers, such as fluorescein and rhodamine. By such "cocapping" experiments, it has been shown that the major histocompatibility loci in man (referred to as HLA–A, HLA–B and HLA–C)[53] and mouse (H–2K and H–2D)[54] code for independent membrane glycoproteins (present on virtually all cells) which are associated with β_2-microglobulin[55] The great majority of macromolecules that have been studied[48,53,54] cap independently, which implies the absence of stable long range ordering of these membrane macromolecules. Moreover, when ligand-induced redistribution is avoided, ultrastructural mapping studies, using ferritin-conjugated antibodies and lectins, generally show a diffuse distribution of antigens and lectin receptors on lymphocytes[56] and other dissociated cells

On the other hand, on cells interacting with each other in tissues or in culture, there is in many cases an uneven distribution of plasma membrane proteins In epithelial cells, certain plasma membrane transport sites[57] and other membrane enzymes[58] are restricted to only one surface of the cell, although they are presumably able to diffuse over large areas of the membrane in which they are confined. More severe restriction of movement exists in the case of gap junctions—those points at which cells in tissues or in culture are ionically (electrically) and metabolically coupled. These junctions are seen in freeze fracture microscopy[59] as polygonal arrays of intramembrane particles; they presumably arise from randomly distributed units in each cell membrane which become highly ordered on cell contact One of the most striking cases of immobile surface proteins is the acetylcholine receptor of the neuromuscular junction. These molecules are confined to the area of the endplate in inervated striated muscle and probably remain fixed even after denervation[60]. The metabolic turnover of gap junction proteins[61] and junctional acetylcholine receptors[62] is extremely slow compared with the turnover of most other membrane proteins[61,63]. This suggests, perhaps, that immobile proteins may be the most stable class of membrane protein.

Although it is unkown how the polarity of epithelial cells is generated, or how junctional acetylcholine receptors are held in place, in principle there are several means by which the distribution and movement of membrane proteins might be controlled These include the formation of large aggregates of protein which could diffuse only very slowly, as is the case in the purple membrane of *Halobacterium halobium*, which is a planar crystalline lattice of membrane proteins[64], and tethering membrane proteins from outside the cell by external fibrous proteins[44], or by cell–cell contact, as in gap junctions.

A quite different mechanism for controlling the distribution and movement of cell surface proteins became apparent with the discovery of antibody-induced capping of surface antigens. The process of capping, and probably also cell locomotion over a surface, involves the directional movement of cross-linked proteins in the membrane whilst allowing free movement of other proteins. Recent evidence suggests that, during capping, those surface proteins which are moved, are tethered to a cytoplasmic matrix which includes microfilaments and possibly microtubules.

Intracellular fibrillar structures in nucleated cells

The three main types of intracellular fibrillar structure seen by electron microscopy in most nucleated mammalian cells are microtubules, microfilaments and intermediate filaments

Microtubules[65], are large, rigid, cylindrical molecules built from a protein subunit called tubulin. Each tubule is composed of 13 parallel 50-Å filaments arranged as a tube of variable length around a hollow 150-Å core. They often form an interconnected cytoskeletal network (through their attachment to centrioles) in the cytoplasm of most mammalian nucleated cells. They are also present in cilia, flagella, and sperm tails where they help produce the motion of those organelles, and in mitotic spindles where they help move chromosomes during mitosis. Cytoplasmic microtubules are usually in a state of rapid assembly and disassembly, processes whose control is still unclear, although Ca^{2+} (ref. 65) and the cyclic nucleotides[66,67] may be involved They can be induced to dissociate by pressure and by cold, and their assembly is inhibited by certain plant alkaloids (such as colchicine and vinblastine) which bind with high affinity to tubulin. Evidence suggests that cytoplasmic microtubules may be involved in controlling membrane protein movement and distribution. For example, the binding of concanavalin A (con A) to specific sugar residues on a cell is known to inhibit antibody-induced redistribution of various cell membrane antigens; this inhibition can be relieved by manoeuvres that disrupt microtubules[68]. This observation has been taken to indicate that many different membrane glycoproteins are linked to the microtubule system[68] As con A is known to bind to a heterogeneous population of membrane glycoproteins, however, it seems possible that it inhibits the movement of otherwise unrestricted lectin receptors by cross-linking them to a subclass of con A-binding glycoproteins which are fixed to the microtubule system[69] Since microtubules as seen by electron microscopy do not interact directly with the plasma membrane of most mammalian cells, their influence on membrane proteins must be indirect, perhaps through microfilaments.

Microfilaments[70] are seen in all nucleated cells as fine filamentous structures with a diameter of about 50 Å. They can be decorated with muscle heavy meromyosin (the active head part of myosin)[70], or with fluorescent anti-actin antibody[71]; this, together with studies on isolated microfilaments, shows that they are made of subunits very similar, if not identical, to skeletal muscle actin. In the cell they are associated with cytoplasmic myosin[72] and tropomyosin[73]. Microfilaments seem to exist as at least two distinguishable species: "lattice microfilaments", which form a loose network of short, interconnected filaments which may be associated with the cytoplasmic side of plasma membranes and which seem to be sensitive to the drug cytochalasin B, and "sheath" or "stress" microfilaments (also called "actin cables")[71] which exist as bundles of fibres running beneath the plasma membrane, often parallel to the long axis of fibroblastic cells. These bundles of microfilaments appear to be less sensitive to cytochalasin B (ref. 70). It is likely that both types of microfilaments are contractile, and collectively have an important muscle-like role in cytoplasmic streaming, various forms of plasma membrane movement, in cell shape changes and locomotion Their possible function in regulating membrane protein mobility is suggested by the demonstration that cytochalasin B (in conditions where its effects on membrane transport are minimised) completely inhibits concanavalin A-induced capping of con A-binding sites on lymphocytes and enables preformed caps to disperse[69]. This suggests that lattice microfilaments have an important role in the capping process, and also in holding the cap at the tail of the cell. There is a hint that, in some circumstances, microfilaments and microtubules may act synergistically in lymphocyte capping; microtubule-disrupting drugs alone do not inhibit capping of immunoglobulin by anti-immunoglobulin antibodies on mouse lymphocytes, and it is only partly inhibited by cytochalasin B (ref. 48), but when cytochalasin B and drugs which disrupt microtubules are used together, they completely inhibit and partly reverse immunoglobulin capping[69] Although caution is required in interpreting these drug effects, it seems likely that microtubular and microfilament networks can cooperate with each other, the former (possibly together with sheath microfilaments) perhaps providing a structural framework relative to which lattice microfilaments and membrane macromolecules can be displaced.

Intermediate filaments[74] are approximately 100 Å in diameter. In neurones they are known as neurofilaments but similar filaments are found in most mammalian cells, often intermingled with microtubules. Their chemistry and function are unknown, and no specific reagents have been shown to disrupt them. Tonofilaments are of similar diameter and are found attached to the plasma membrane of epithelial cells at specialised sites called desmosomes.

At present, the intense interest in these intracellular fibrous proteins is aimed at understanding their structure and function. One would like to know the nature and extent of their heterogeneity and interactions, how they interact with cell membranes, and the factors that control their orientation within the cell, their assembly and disassembly.

Intramembrane particles in nucleated cell plasma membranes

In nucleated mammalian cells, such as lymphocytes or fibroblasts, freeze fracture electron microscopy reveals intramembrane particles more heterogeneous in size and less densely distributed than in erythrocytes This greater heterogeneity presumably reflects the large variety of globular proteins in, and thus the greater biochemical complexity of, these membranes. Furthermore, unlike in erythrocytes, most attempts to demonstrate a relationship between the particles and specific cell surface receptors and antigens have failed. Thus, when surface antigens or lectin receptors are capped on lymphocytes[75], or when lymphocyte surface proteins are covalently labelled with haptenic groups and these are capped with anti-hapten antibody[76], no equivalent redistribution of the intramembrane particles is seen. Moreover, when lymphocytes or fibroblasts are exposed to high concentrations of glycerol, which reversibly aggregates the particles in these cells[77], no concomitant aggregation of con A–ferritin-binding sites is seen by electron microscopy (N. B. Gilula, D. Lawson and M C R., unpublished). Thus, it seems that the majority of proteins on the surface of these cells that bind antibodies or lectins, or that can be readily haptenised, are not associated with the particles.

This inability to identify particles with external receptors and antigens probably results from the existence of two different classes of surface proteins in nucleated cells Both may span the plasma membrane, but they are otherwise distinguished in the following ways. One group has an extensive region of the polypeptide and attached oligosaccharide exposed on the outer surface of the cell but little mass in the plane of the bilayer, as in the case of the erythrocyte MN glycoprotein. These surface components would be expected to bind ligands well, but, by themselves, would not be seen in the freeze fracture plane because their intramembrane mass is too small. What happens to such a protein during freeze cleavage? Either one or other region of the polypeptide chain on either side of the membrane pulls out of the ice with which it is in contact, or covalent bonds in the protein are broken. Something must give. For example, it is known that in Semliki Forest virus the spike proteins span the viral membrane[78], yet no particles are seen in the fracture plane It seems possible that in this case, and possibly many others, the covalent backbone of that region of the protein inside the membrane has been ruptured. Proteins of the other class are globular, have most of their mass in the plane of the membrane and thus are seen as particles, but do not extend appreciably outside the external surface of the plasma membrane bilayer and are therefore rather inaccessible to ligands. The major erythrocyte protein (of 100,000 daltons, band 3) seems to have these properties; it is remarkably unreactive on its external surface[20] and would thus be difficult to haptenise. In other plasma membranes, haptenisation of similar globular proteins may also prove to be difficult; an attempt to aggregate them by anti-hapten antibody might therefore be unsuccessful and leave them (and thus the particles) unmoved. It seems likely that this latter class of globular membrane protein would include the many proteins involved in the active and passive transport of small molecules (such as ions, sugars, nucleosides and amino acids) and in the formation of cell junctions.

Most of the globular proteins associated with transmembrane functions are difficult to purify and study biochemically. The great value of the freeze fracture technique is that it shows us the distribution of these proteins in the plane of the membrane with remarkable clarity. What would improve its power enormously would be some general method for relating components, identified either as a band on a gel or by serological methods, to the particles seen in the freeze fracture plane of a membrane. Freeze fracture autoradiography and defining particle subclasses by improved shadowing techniques should prove extremely useful in this respect (D Branton, personal communication).

Membrane carbohydrate

The proportion of carbohydrate in plasma membranes is never more than 10% by mass and often much less. It is present mainly as oligosaccharide side chains covalently attached to proteins and, to a lesser extent, sphingolipids. Although more than one hundred different monosaccharides are found in nature, only about ten occur in membrane glycoproteins and glycolipids, the principal sugars are galactose, mannose, fucose, galactosamine, glucosamine, glucose and sialic acid[79], the last usually being terminal and the chief contributor to the net negative surface charge carried by all mammalian cells Glycolipids and glycoproteins contain similar sugars, except that in glycolipids, mannose is generally absent and glucose is usually found as the first sugar linked to lipid The oligosaccharide side chains of membrane glycoproteins, and of a small number of glycolipids, can be remarkably complex Although they are rarely more than 15 sugar residues long, they are usually branched, and can be bonded together in a variety of different linkages[79]. This could give rise to a great range of structures so that, for example, three different sugar molecules can be arranged into hundreds of different trisaccharides. Although substantial progress has been made in elucidating the sequences of many oligosaccharides bound to lipid or protein, the biological functions of the carbohydrate remain obscure. In both internal and plasma membranes the carbohydrate seems to be located exclusively on the non-cytoplasmic surface[18,80] and several functions have been proposed to take this restricted location into account.

At one level, carbohydrate is regarded as important for membrane biosynthesis[20]. It is likely that in nucleated cells all membrane proteins are initially incorporated into the rough endoplasmic reticulum. From there, they diffuse, or are conveyed, to their different membrane locations within the cell—the smooth endoplasmic reticulum, nuclear membrane, Golgi apparatus or plasma membrane. But the detailed mechanism for how these proteins are initially placed in the rough endoplasmic reticulum is unknown; it is often supposed that this is done during synthesis by secretory ribosomes bound to the endoplasmic reticulum, implying that membrane proteins are a special class of secreted protein. The alternative view[20] is that membrane proteins are initially synthesised as soluble proteins which dissolve into the endoplasmic reticulum from the cytoplasm. To "lock" these proteins into the membrane, they are glycosylated from the lumen side of the endoplasmic reticulum by a large number of different glycosyl transferases, which are specific for the sugar, acceptor site and the linkage catalysed. This scheme places the onus of anchoring those proteins (which span the membrane) on the carbohydrate. Without this "lock", the protein could diffuse back into the cytoplasm, a situation which would make it much more difficult for a cell to maintain specific proteins in specific membranes. In bacteria, in which only one membrane (the plasma membrane) exists, no such problem arises and membrane proteins are generally not glycosylated. Once in the rough endoplasmic reticulum, proteins destined for the plasma membrane move to the Golgi complex where more extensive glycosylation occurs; finally, they pinch off the Golgi apparatus in vesicles which fuse with the plasma membrane[81].

But the idea that carbohydrate acts as a "lock" on those proteins which span the membrane does not explain the observed complexity of carbohydrate sequences A simple oligosaccharide (such as penta-sialic acid) would do as a "lock", without any need for the wide variety of oligosaccharide chains which exist. One is therefore inclined to look further for additional functions. The potential of complex oligosaccharide labels for the purpose of specific cell–cell recognition has not escaped notice[82], and it seems possible that they could have such a role The indirect nature of the genetic control, however, and the resulting variability in the extent of glycosylation, could allow a worrying amount of degeneracy if such a system were used for fine specificity in cell marking. Nevertheless, the consistent differences in oligosaccharide side chains between and among the different glycolipids and glycoproteins indicate the existence of important mechanisms for controlling their glycosylation.

The terms "cell coat" or "glycocalyx" refer to the carbohydrate-rich peripheral zone found at the surface of all mammalian cells, which can be visualised by a variety of stains more or less specific for carbohydrate, such as ruthenium red[83]. The carbohydrate consists of the oligosaccharide side chains of plasma membrane glycolipids and glycoproteins, including in some cases, adsorbed glycoproteins and mucopolysaccharides (proteoglycans). This high concentration of cell surface carbohydrate must have an important influence on many of the functions of the plasma membrane, but the nature of these influences is poorly understood. The use of highly purified glycosidases and mutant cell clones deficient in certain cell surface monosaccharides[84] should prove useful in this respect

Conclusions

All biological membranes, including mammalian plasma membranes, are structurally based on a lipid bilayer. The strongest evidence for this comes from the susceptibility of the membranes to fracture across their mid-planes at low temperature. The bilayer can be visualised as a two-dimensional fluid, with the individual lipid molecules asymmetrically distributed between the two leaflets and able to diffuse in the plane of their own monolayer. Into this lipid matrix, which provides the basic structure of the membrane, specific proteins are inserted asymmetrically and these are responsible for most of the membrane's functional properties. In the case of the plasma membrane, these functions include the receiving and transducing of various extracellular chemical signals (such as hormones, growth factors, neurotransmitters, antigens), cell junction formation (gap, tight, and synaptic), transport of small molecules, endocytosis (pinocytosis and phagocytosis) and exocytosis. Some of the membrane proteins span the bilayer, either as a simply coiled hydrophobic segment (as in the erythrocyte MN glycoprotein) or in a more folded globular state. Most, if not all, of these are glycosylated, having all of the carbohydrate on the non-cytoplasmic side, which probably serves to lock the proteins in place. Since there is no evidence in the erythrocyte (or any other) plasma membrane for a protein which is inserted solely into the outer monolayer of the membrane, it is likely that every membrane glycoprotein spans the bilayer. It seems probable that most of the polypeptides exposed on the cell surface which do not span the membrane (for example, β_2-microglobulin) are held in place by association with other polypeptides which do so.

In human erythrocytes, the major glycoproteins which span the membrane probably comprise the majority of the intramembrane particles seen in the fracture plane; they do not turn over (if lost, they could not be replaced) and their in-plane mobility is markedly restricted, probably because they are tethered from within by spectrin. In contrast, in nucleated cells, most of the readily detectable surface-exposed plasma membrane proteins seem not to be associated with intramembrane particles, are rapidly turning over and are mobile in the membrane plane; on the other hand, membrane proteins interacting between nucleated cells at sites of specialised intercellular junctions often appear to be associated with particles, are restricted in translational mobility, and turn over comparatively slowly. Little is known about the in-plane mobility or turnover of non-junctional proteins seen as particles. In addition, there is evidence that the cytoplasmic fibrous proteins of nucleated cells, such as microfilaments and microtubules, can control the distribution and translational movement of some membrane proteins.

There are obvious advantages in having a fluid membrane in which molecules can diffuse freely. It may enable membrane enzymes and their lipophilic substrates to come together and interact, and it provides a simple means of distributing proteins and lipids to regions of the membrane remote from where they are inserted, and for dividing membrane components evenly between daughter cells at the time of cell division. In addition, it would be expected to facilitate cell locomotion and membrane fusion, and allow a more adaptable fit between receptors and multivalent ligands or other cells. The alternative of having a frozen and thus rigid lipid bilayer could be dangerous to the cell, as a slight deformation might crack the membrane and lead to the loss of cytoplasmic components On the other hand, in some circumstances (such as during locomotion), a mechanism for controlling the distribution and mobility of membrane proteins within the fluid milieu is essential. This balance between freedom and control is seen in all things, not least of which is the mammalian plasma membrane.

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articles

Mapping of the G_1 phase of a mammalian cell cycle

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Temperature-sensitive variant clones of BALB/3T3 cells were put on a linear time scale map by measuring their time of entry into S phase; these clones seemed to accumulate a unique non-histone protein at the restricted temperature.

THERE is an extensive literature on the biochemistry and control mechanisms of the four phases of the cell cycle[1] (for reviews see refs 2 and 3); but little is known of the biochemical events in G_1 (between completion of mitosis and onset of DNA synthesis) and G_2 (between completion of DNA synthesis and mitosis). The production of RNA and proteins involved in the initiation, progress and termination of DNA synthesis are some of the main events attributed to G_1 and G_2 (refs 2 and 3); however, most of the evidence was indirect since selective inhibitory agents (for example, dactinomycin) were used. Other investigators[4] have referred to the resting (in respect of proliferation) cells as being in G_0, distinct from G_1, which can be stimulated to divide by an appropriate stimulus[5]. Such non-growth states can be achieved experimentally and attention has focused recently on cells so arrested in G$_1$ as potential "target cells" for carcinogenic transformation[6,7]. It has also been suggested that there are some rate controlling signals in G$_1$ itself and at the G$_1$/S boundary which have regulatory functions[8–10]. We have mapped G$_1$ of a mammalian cell cycle using temperature-sensitive variant cell lines. There have been several reports of variant clones defective in G$_1$ (refs 11–15), but for a comprehensive study a linear time scale map is essential, and this article presents data on such a map.

An aneuploid clone (A31) of BALB/3T3 (ref. 16) was obtained from Imperial Cancer Research Fund Laboratories, London. The culture was maintained in L-15 medium[17] with 10% foetal bovine serum (FBS), penicillin (100 U ml^{-1}) and streptomycin (50 µg ml^{-1}) at 33 °C. Temperature-sensitive variant clones were maintained in similar conditions. A preliminary study of the generation time of wild-type BALB/3T3 cells showed that between 33 °C and 38 °C the cells doubled at approximately the same rate (21±0.5 h and 20±0.75 h, respectively). Replicate cell cultures plated at 1,000 per Falcon tissue culture plates (60× 15 mm) at 38 °C were exposed to doses of N-methyl-N′-nitrosoguanidine (MNNG) (Aldrich, 05 µg ml^{-1}) every 90 min for 9 h after plating. This was done to catch cells at different stages of G$_1$ in an asynchronous population. After 2 h of treatment with MNNG (lethality less than 50%) cells were exposed to either 2.5×10^{-4} M 5-bromo-2-deoxyuridine (BUdR, Sigma)[18] or 6-^{3}H-thymidine[19] (5 µCi ml^{-1}, 32 Ci mmol^{-1}) for 2 h in a prewarmed medium at 38 °C to kill the DNA-synthesising cells selectively. The culture plates were then washed in normal growth medium (L-15) with 10% FBS and incubated at 33 °C until discrete clones were visible. Half the medium was replaced every 3 d Isolated clones were purified further from single cell colonies through two successive reclonings. Forty-five temperature-sensitive clones were isolated after preliminary selection in this way. Of these, eight were studied because they were relatively the least 'leaky'—A8, A69, A83, A90, A92, A122, A123 and B23.

Table 1 Colony-forming ability of BALB/3T3 and temperature-sensitive variants at 33 °C and 38 °C after 7–10 d of incubation

Clones	No. of input cells per 25 cm^2 Falcon tissue culture flasks							
	5×10^2 cells		1×10^3 cells		5×10^3 cells		1×10^4 cells	
	33 °C	38 °C	33 °C	38 °C	33 °C	38 °C	33 °C	38 °C
Wild type	56±3	61±7	82±7	116±4	c	c	c	c
A8	29±3.3	0	71±5	0	c	26±8	c	73±11
A83	33±4	0	59±10	0	c	32±13	c	92±14
A90	30±6	0	58±12	0	c	45±18	c	108±22
A92	23±1	0	63±2 8	0	c	92±15	c	c
A122	59±5	0	117±4	0	c	97±7	c	c
A123	63±8	0	123±12	0	c	58±12	c	138±8
B23	18±4.6	0	40±5.5	0	c	106±10	c	c

To determine colony-forming ability, trypsinised cells were plated in 25 cm^2 Falcon tissue culture flasks at a density of 10,000, 5,000, 1,000 and 500 per flask in L-15 medium Replicate cultures were incubated at 33 °C and 38 °C for 7–10 d. Half the medium was replaced every 3 d. Finally, the cultures were stained in Giemsa. c, Confluent culture (> 250 colonies).

These variant clones differed (4–12%) in their ability to form colonies at 33 °C when plated at 1,000 and 500 cells per flask, but almost no colony grew in any of them at 38 °C at these densities (Table 1). Compared with the variant cells, the parental cell line had more colonies growing at 38 °C (11.6%) than at 33 °C (8 2%). At greater densities (5,000 and above) variant clones would grow and appeared more and more leaky at the restricted temperature (38 °C). Temperature shift-down after 10 h at 38 °C revealed no significant decrease in colony formation in any temperature-sensitive variant. This suggested that the temperature-sensitive defects were at least partly reversible, but were irreversibly impaired during prolonged incubation at 38 °C. To pinpoint the phase of the cell cycle when the temperature-sensitive cells were defective we compared the cell cycles of wild-type BALB/3T3 and temperature-sensitive variants at 33 °C and 38 °C. In general we followed Baserga's[20] method. We counted labelled cells in mitosis in 200 metaphase plates of each sample. The labelled metaphases represented cells that had gone through G$_2$, mitosis, G$_1$, S, second G$_2$ and mitosis after a pulse label. The average generation time was estimated from the difference between 50% points on the two ascending limbs of the biphasic curve[20,21]. Mitotic index was calculated from the same preparations by counting the average number of cells in mitosis in 50 microscopic fields. The data for two temperature-sensitive clones are presented here for comparison with the parental cell line. The division cycle of cells showed (Fig. 1*a*–*c*) that the duration of one cell cycle in wild-type BALB/3T3 (Fig. 1*a*) was approximately 20.5 h at both 33 °C and 38 °C, but the distribution of the four phases differed slightly At 38 °C, the durations of phases were G$_1$+M, 6 5 h; S, 10.5 h, and G$_2$, 3.5 h, whereas at 33 °C they were G$_1$+M, 8.5 h; S, 9.5 h; and G$_2$, 2.5 h. In the variant clones A8 (Fig. 1*b*) and A83 (Fig. 1*c*), however, S seemed to be slightly longer at 33 °C, but at 38 °C both clones failed to reach S. Labelled cells in mitosis did not exceed 50% in the variant clones at 38 °C. This was true for all eight variant clones.

Mitotic indices counted from the same preparations showed (Fig. 1*a*–*c*) that at 33 °C the variant clones A8 and A83, behaved very similarly to the parental cell line, the mitotic index increasing from 1–2% to about 4.8%. At 38 °C, however, mitotic indices were significantly different. The parental culture and clone A8 had mitotic indices more or less indentical to those at 33 °C; in clone A83, on the other hand, the mitotic index rose from 1.6% to 48.8% between 12 and 24 h at 38 °C. The latter was at first thought to indicate cells arrested in mitosis, similar to the situation with colchicine treatment, but an analysis of the cells at different stages of mitosis (prophase : metaphase : anaphase : telophase) was more or less similar between 12 and 24 h (1.6: 5.6: 1.0: 1.5 respectively). If anything there was an accumulation of cells in anaphase and telophase during the later stages of incubation of A83 at 38 °C. This may represent a slowing down of 'transit' through all phases of mitosis. We checked this by testing the viability of the cells arrested at 38 °C by means of temperature shift-down (from 38 °C to 33 °C). We found that the longer the cultures were incubated at 38 °C, the larger the number of cells entering S phase, though they were delayed This indicated that many A83 cells arrested at 38 °C remained viable for the next 10–12 h. The variants were mapped by timing the start of DNA synthesis For this purpose, 1×10^5 cells per ml were seeded in Leighton tubes on coverslips in 1 ml volumes as before The culture tubes were incubated for one generation time (20 h) at 33 °C; thereafter the cultures were transferred to 38 °C except for one set of controls. Replicate culture tubes (in triplicate) were incubated at 38 °C for 2, 4, 6, 8 and 10 h and transferred back to 33 °C for 8, 6, 4, 2 and 0 h, respectively. From the 10th hour, culture tubes from each set of transfers were pulse labelled for 10 min with 6-^{3}H-thymidine (5 μCi ml^{-1}, 32 mCi mmol^{-1}) at 33 °C every hour for 12 h. Preliminary studies of the cell cycle of the parental line (Fig. 1) showed that the cells

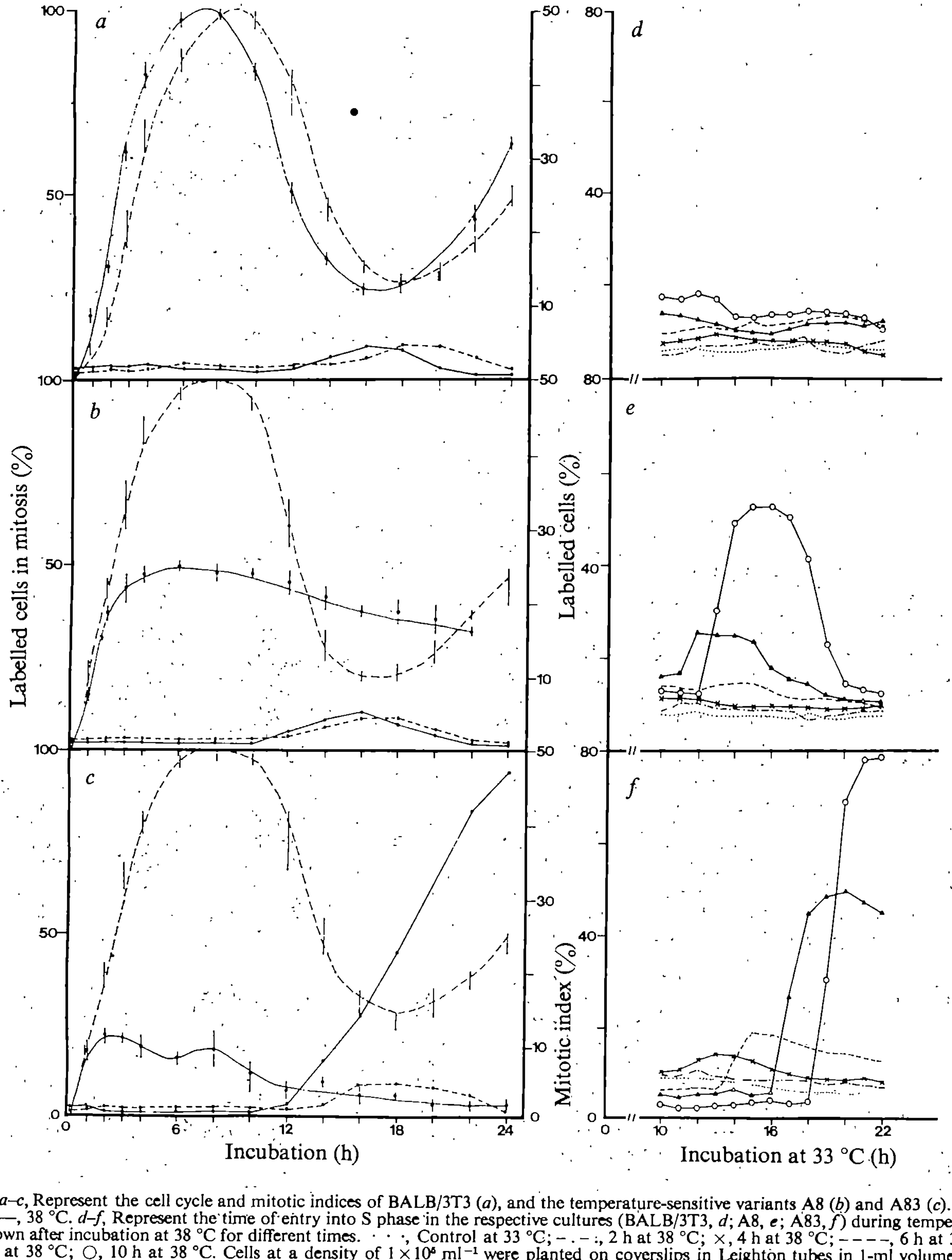

Fig. 1 *a–c*, Represent the cell cycle and mitotic indices of BALB/3T3 (*a*), and the temperature-sensitive variants A8 (*b*) and A83 (*c*). – – –, 33 °C; —, 38 °C. *d–f*, Represent the time of entry into S phase in the respective cultures (BALB/3T3, *d*; A8, *e*; A83, *f*) during temperature shift-down after incubation at 38 °C for different times. · · ·, Control at 33 °C; – . – ., 2 h at 38 °C; ×, 4 h at 38 °C; – – – –, 6 h at 38 °C; △, 8 h at 38 °C; ○, 10 h at 38 °C. Cells at a density of 1×10^5 ml^{-1} were planted on coverslips in Leighton tubes in 1-ml volumes and incubated for 36 h at 33 °C to enable the cells to reach the log phase of growth. Cells were then exposed to 6-^{3}H-thymidine at a concentration of 1 µCi ml^{-1} (5 Ci mmol^{-1}) for 10 min and washed in Hanks' balanced salt solution (BSS) containing cold thymidine (5 µg ml^{-1}) to dilute the remaining radioactivity in the cells. Finally, the cells were placed in L-15 medium containing cold thymidine (5 µg ml^{-1}); replicate tubes were incubated at 33 °C and 38 °C. At hourly or two-hourly intervals one set of tubes was fixed, autoradiographed and stained by the following method. After removing the medium, cells were treated with a hypotonic solution of Hanks' BSS in water (1:4) for 5 min, fixed for 5 min in acetic acid: ethanol (1:3) diluted in the hypotonic solution, and then fixed for a further 10 min in undiluted fixative. The coverslips were air dried and mounted in glass slides with the cells on the upper side. The slides were then dipped in Ilford L-4 liquid emulsion and exposed for 2 weeks in the dark at –20 °C. After 2 weeks the slides were developed in D19 developer, dried and stained in 4% buffered Giemsa.

entered S between 8.5 and 10 h. The design of our experiments was expected to enable the parental cell line to enter without delay; but a fractional population with a defect in G_1 in the variant cell line would be delayed in its biochemical processes and would thus enter S late. The time a variant culture was delayed in entering S was calculated to be the time (map distance) from the S phase with respect to the parental culture. This map of G_1 variants is strictly a measure of their time of entry into S; it is not a complementation or genetic map.

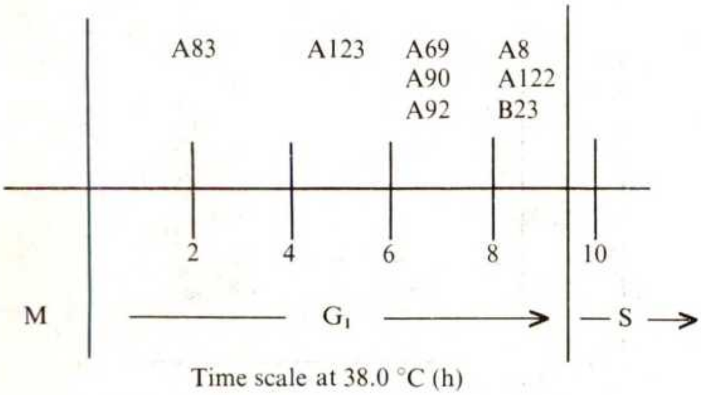

Fig. 2 Tentative map of the G_1 phase of the cell cycle of temperature-sensitive clones of BALB/3T3 cells, measured with respect to the time of the block and entry into S phase.

The coverslips from each tube after exposure to radioactivity were washed, fixed and autoradiographed as in the cell cycle experiment. The percentage of labelled cells was counted from 50 microscopic fields; cells in active DNA synthesis were counted for 12 h. The results showed (Fig. 1*d*–*f*) that in the wild type parental culture (Fig. 1*d*) there was no G_1 accumulation of cells entering S during successive transfers from 38 °C; if anything, the percentage of labelled cells was slightly higher in the last two transfers at 8 h (9.6%) and at 10 h (14.6%), indicating more cells already entering S at 38 °C. The number of labelled cells, however, decreased as the culture progressed through the growth cycle at 33 °C. The variant clones A8 (Fig. 1*e*) and A83 (Fig. 1*f*), on the other hand, had presumably accumulated cells in G_1 during longer periods of incubation at 38 °C so that the cells were delayed in entering S for different times; this was reflected in the larger number of labelled cells appearing in later transfers. In the variant clone A8, the delay in entering the S phase and consequent accumulation of labelled cells appeared between 8 and 10 h incubation at 38 °C, whereas in clone A83 the delay and accumulation of cells entering S were first observed between 2 and 4 h incubation at 38 °C. It is interesting to compare the corresponding increase in the percentage of labelled cells in the variant clones during longer periods of incubation at 38 °C. In clone A8, the number of labelled cells increased from 16.6% to 25.8% after 8 h of incubation at 38 °C (Fig. 1*e*); but after 10 h at 38 °C the number of labelled cells increased from 12.0% to 52.2%, though entry into S was delayed by 2 h in the latter case. The same appeared to be true for A83. After 4 h of incubation at 38 °C (Fig. 1*f*) cells started to accumulate in G_1, denoted by a corresponding delay in entering S at 33 °C. The percentage of labelled cells among those transferred after 8 h increased from 5.9% to 58.4%; after 10 h at 38 °C it increased from 3.8% to 78.4%. These results indicated that the variant cultures attained 'partial synchrony' in entering S phase because they had longer periods of incubation at the restricted temperature (38 °C); the cells were presumed to be arrested in G_1.

The 'partial synchrony' was soon lost after the cells were shifted to 33 °C. In both A8 and A83, 'synchronised' populations began to lose synchrony within 3–4 h at 33 °C; the percentage of labelled cells decreased sharply instead of forming a plateau. Since the measured time for S phase was between 10 and 12 h at 33 °C, the synchrony was probably lost halfway into the S phase in these variant cell lines.

The duration of the S phase in the parental and variant (A8, A83) cultures being about 10 h at 33 °C (half the generation time for each cell), at least 50% of cells would be expected to be labelled with ^{3}H-thymidine at any time in the control cultures, but we observed only 10–20% labelling of cells at 33 °C. This was thought to be due to either (1) lower efficiency of plating (<50%) in these cells, or (2) many cells not actively cycling in these early log phase cultures incubated for 20 h at 33 °C. This series of experiments was conducted for each of the other six variant clones. On the basis of their time of entry in S, the 'mutant' cells were tentatively put on a linear time scale map (Fig. 2). (The final assignment of these 'mutant' clones on a genetic map awaits completion of complementation tests now in progress.)

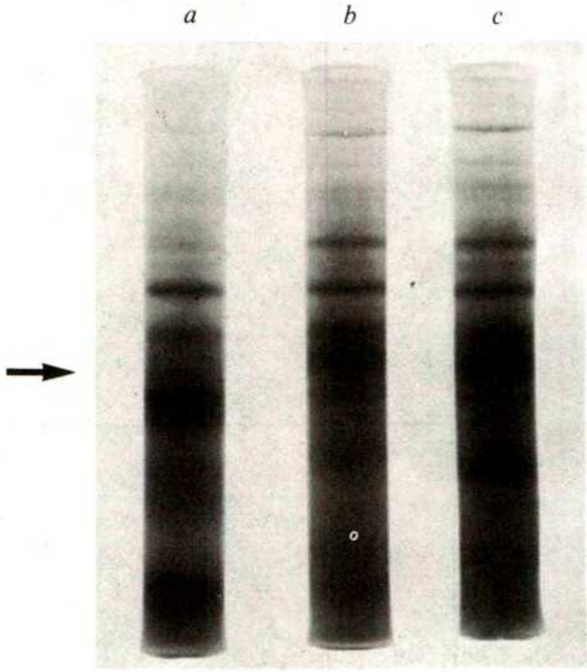

Fig. 3 Polyacrylamide gel electrophoresis of 100 µg of nuclear proteins of cultures, at 38 °C, of parental cell line of BALB/3T3 (*a*), clone A8 (*b*) and clone A83 (*c*). Cells at a density of 1×10^{-5} ml^{-1} in 30 ml volumes were plated in 16-ounce glass bottles and incubated for 16 h at 38 °C. Cultures were collected from the bottles after two washes with cold phosphate-buffered saline (PBS) in 1 ml of 1% Nonidet P40. Cells were then washed with PBS and frozen. Nuclear proteins were prepared by the methods described in Fig. 4. Gels were stained with 2% naphthalene black.

We analysed the nuclear proteins in the two variant clones (A8, A83) and the parental clone, grown for 16 h at the restricted temperature (38 °C) to identify possible biochemical alteration in the nuclei of the variants at the restricted temperature. Nuclear proteins were prepared as before[22]. Equal amounts (100 µg in 0.2 ml) were run on 6% acrylamide disc gels with sodium dodecyl sulphate in a continuous system[22]. Stained preparations of these gels revealed

(Fig. 3) a unique protein in both variant clones (Fig. 3). On the other hand, several low molecular weight proteins at the bottom of the gel in the parental type were absent from the variant clones. There was, however, similarity in the electrophoretic profiles of the nuclear proteins in the two variant clones which are widely separated in our time scale map of G_1.

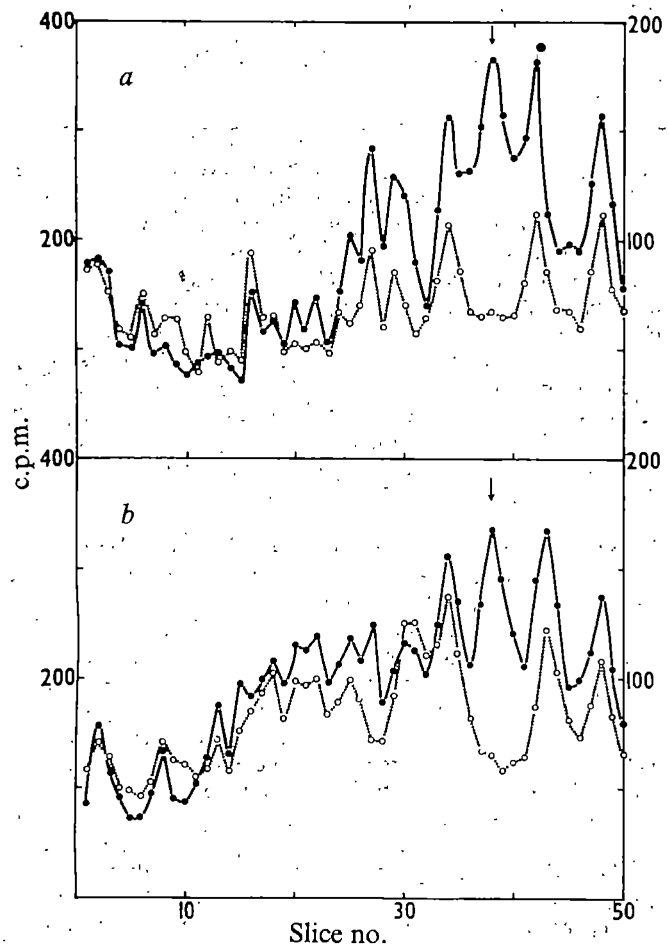

Fig. 4 *a*, Distribution of ^{3}H-tryptophan (●) and ^{14}C-methionine (○) in 100 μg of nuclear protein of clone A83. Cells were incubated for 16 h at 38 °C and double labelled with radioactive amino acids for 1 h at 38 °C. *b*, Distribution of ^{3}H-tryptophan (●) and ^{14}C-tryptophan (○) in coelectrophoresis of 100 μg of nuclear proteins of clone A83 grown at 38 °C and 33 °C respectively for 16 h and exposed to radioactive medium for 1 h at their respective temperatures. Quantitation of the nuclear proteins was performed as follows. Cells at a density of 1×10^5 ml^{-1} in 30 ml volumes were plated in 16-ounce glass bottles and incubated for 16 h at 38 °C. The cells were then washed with Hanks' BSS and exposed to Hanks' BSS with L-methionine-methyl-^{14}C (10 μg ml^{-1}, 56 mCi mmol^{-1}), or L-(methylene-^{14}C)-tryptophan (10 μg ml^{-1}, 2Ci mmol^{-1}), and L-tryptophan-^{3}H-G (24 μg ml^{-1}, 5 Ci mmol^{-1}) for 1 h at 38 °C. Cultures were collected from the bottles after two washes, with cold PBS, in 1 ml of 1% Nonidet P40. Cells were then washed with PBS and frozen. Frozen cell pellets were washed and resuspended in rabbit reticulocyte standard buffer (RSB, *p*H 7.4) and allowed to swell for 10 min at 2 °C. The cells were homogenised on a Dounce homogeniser, using 10–15 strokes. In these conditions, 95–100% of cells released intact nuclei and cytoplasm. Nuclei were centrifuged at 500*g* for 15 min and washed with RSB. The nuclear pellets were resuspended in RSB (10^7 nuclei ml^{-1}) and disrupted by sonication for 5 min at full power in a Soniprobe Type 7530A sonic oscillator. The nuclear fraction (100 μg in 0.2 ml) was first reduced by treating it with 0.5 ml of a mixture of 10 M urea, 4% SDS and 4% mercaptoethanol and heating at 100 °C for 3–5 min. The sample was run on 6% acrylamide gels with SDS in a continuous system. The distribution of radioactive proteins in the gels was determined by slicing the gel into 1-mm thick sections, dissolving it in 0.5 ml of H_2O_2 at 60 °C and counting in toluene scintillation fluid (toluene–Triton, 1:1).

For a qualitative and quantitative estimation of the different proteins in the gels, the variant A83 cells were labelled and the radioactivity in the nuclear proteins was analysed. The distributions of radioactive proteins, doubly labelled at the restricted temperature with ^{14}C-methionine and ^{3}H-tryptophan showed (Fig. 4*a*) a high rate of incorporation of tryptophan in the band indicated. Since tryptophan is incorporated selectively into non-histone proteins, we presume that this fraction is a non-histone protein. To acquire further evidence that this protein accumulated only at the restricted temperature equal amounts of nuclear proteins (100 μg) of ^{3}H-tryptophan-labelled A83 cells at 38 °C and ^{14}C-tryptophan-labelled A83 cells at 33 °C were run together; they did not band in the same molecular weight region (Fig. 4*b*). Appearance of an identical non-histone protein in the two variant clones, A8 and A83, defective in G_1 suggests that these variant clones were accumulating a protein that was not involved in turnover at 38 °C. Isolation and characterisation of this protein has now been undertaken.

Our results show that temperature-sensitive cell lines could provide a useful tool for study of the control of the cell cycle in mammalian cell systems, particularly in the identification of endogenous components necessary for the regulation of cell growth. There are increasing indications that differentiation and carcinogenesis are related to regulatory changes in a cell[23,24] which are possibly determined during G_1. A tendency for different mammalian cell types to have G_2 and M of similar durations but a G_1 of widely varying durations has been offered repeatedly as evidence that controlling events occur in G_1 (refs 20, 25 and 26). A significant conclusion from our experiments is that some of the events controlled in G_1 are apparently sequential with respect to their biochemical events. Arresting any of these events delayed the subsequent processes.

The molecular mechanism involved in the alterations of a particular enzymatic function in the temperature-sensitive cells is not well understood. Whether the variant clones in a conditionally defective state (at the restricted temperaure) make an altered enzymatically inactive protein ("missense") or no protein at all ("nonsense") has yet to be resolved. To what extent these structurally altered proteins could be corrected, and the time it would take for the correct resolution might to a certain extent jeopardise a linear time scale map over short sequences, but over long sequences such a map would probably provide a good foundation for the study of functions controlled in G_1.

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Nuclease that preferentially inactivates DNA containing mismatched bases

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Heteroduplex and homoduplex DNA molecules of bacteriophage SPP1 were treated with DNase I, an enzyme of Ustilago maydis *implicated in genetic recombination. A transfection assay with* Bacillus subtilis *demonstrates that the heteroduplexes are preferentially inactivated.*

It is widely accepted that hybrid or heteroduplex DNA is an essential intermediate in genetic recombination[1-5]. In heterozygous crosses, the inclusion of a genetic marker within a hybrid DNA segment will result in the formation of a mismatched base pair. It was suggested that gene conversion (non-reciprocal allelic recombination) could be due to the repair of such mismatched bases[6,7]. Genetic evidence for such repair with artificially made heteroduplexes has subsequently been obtained in transfection experiments with bacteriophage SPP1 of *B. subtilis*[8], in phage λ[9,10], as well as in transformation with *Pneumococcus*[11] and studies on gene conversion and post-meiotic segregation in *Ascobolus*[12]. An initial step in the repair of mismatched bases is presumed to involve an enzyme that cleaves one strand of the DNA at or near the mismatched region, analogous to the initial step in pyrimidine dimer excision repair.

A search for such an enzyme in the fungus *Ustilago maydis* was initiated by the isolation of DNase deficient mutants, which were also defective in the process of gene conversion[13,14]. The deficiency of gene conversion was shown to be associated with a low level of a nuclease, DNase I[15]. We show here that this enzyme preferentially inactivates heteroduplex molecules containing a single mismatched base pair or a deletion, as well as molecules containing pyrimidine dimers.

Ustilago nuclease and SPP1 transfection

The enzyme has been purified 8,600-fold from wild-type extracts and has been fully characterised[14-16]. Like the related nucleases of *Neurospora crassa*[17] and *Aspergillus nidulans*[18], it is most active on single-stranded DNA substrates. Although it does not degrade native DNA to acid soluble products, alkaline sucrose gradient analysis showed that single-strand nicks are introduced in such molecules. Previous irradiation of the substrate with ultraviolet light increases the number of nicks introduced, which indicates that the enzyme recognises minor distortions in DNA. This activity on native DNA is not due to a second contaminating nuclease. The enzyme is unusual in having no activity on circular DNA, whether single or double stranded. Free ends of DNA molecules are therefore essential for its activity.

Native DNA from phage SPP1 is taken up by competent cells of *B. subtilis*, which later produce a burst of phage particles[19]. The strands of SPP1 DNA after complexing with RNA can readily be separated in CsCl density gradients. Separated complementary strands obtained from mutant and wild-type phages can be reannealed to form biologically active homoduplex and heteroduplex molecules[8,20]. It is known (personal communication from H. C. Spatz and T. A. Trautner), that a single-strand nick inactivates the molecule in the transfection assay. The availability of defined homoduplex and heteroduplex molecules provides a very sensitive substrate for any enzyme which specifically attacks heteroduplex molecules.

Hydroxylamine-induced (G–C→A–T) plaque-type mutants have been isolated in SPP1 phage. Most of the experiments involve one of these, mutant 161. The mismatches in heteroduplex DNA therefore involve single bases, the pairs being either adenine–cytosine or guanine–thymine. Many deletion mutants have been isolated. We have used one, 4307, which lacks up to 3% of the genome, as judged by its buoyant density and its sensitivity to citrate inactivation (personal communication from G. Klotz and T. A. Trautner).

Inactivation of hetero- and homoduplex DNA

The two heteroduplexes, two homoduplexes and native DNA were exposed to *Ustilago* nuclease for 15 min under standard assay conditions (see legend to Table 1). Competent cells were added and incubated for 30 min at 37 °C. The cells were then diluted and plated to score the number of infective centres.

The difference between the inactivation of homoduplex and heteroduplex substrates was about sixfold in the experiment in Table 1; in all experiments it varied between three- and sevenfold. Nevertheless, significant inactivation of native and homoduplex was seen, which could be due to contaminating nuclease activity. This possibility, however, seems very unlikely on the basis of results from experiments done to dissociate the nonspecific activity (on homoduplex or native DNA) from the specific activity (on heteroduplex DNA). In experiments in which the salt concentration of the reaction mixture was varied, or different dilutions of the enzyme were used, reduction or loss in specific activity led to a simultaneous reduction or loss of the nonspecific activity. It therefore seems probable that a proportion of the native and homoduplex molecules are imperfect structures. The process of hybridisation may increase the proportion of defective molecules and therefore tend to minimise the difference between homoduplex and heteroduplex

Table 1 Inactivation of native, homoduplex and heteroduplex DNA by DNase I

Type of DNA		Infective centres No enzyme	Infective centres + enzyme	% Surviving Biological activity
Native SPP1		2.4×10^5	1.2×10^5	47.6
161/161	homoduplex	1.6×10^4	4.0×10^3	24.5
+/+		2.7×10^4	7.2×10^3	26.6
161/+	heteroduplex	3.3×10^4	1.2×10^3	3.6
+/161		2.1×10^4	8.2×10^3	3.9

The *Ustilago* enzyme was purified according to the procedure of Holloman and Holliday[15], and fraction V was used in all experiments. A solution containing 7,000 U ml^{-1} was diluted 1/10 in 10 mM Tris (*p*H 7.5) and 5 μl was incubated with 0.1 ml DNA (5 μg ml^{-1}; saturating conditions in transfection) and 0.1 ml of 10 mM $MgCl_2$ for 15 min at 37 °C. The reaction was terminated by adding 0.2 ml competent cells (a dilution effect). After addition of competent cells the standard transfection procedure was followed[19]. Each number represents infective molecules per ml, individual values being obtained from a total plaque count of ~ 600–800.

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Table 2 The effect of DNase I on deletion heteroduplex and homoduplex DNA

Type of DNA		Infective centres No enzyme	Infective centres + enzyme	% Surviving Biological activity
4307/4307		2.5×10^3	1.7×10^3	70.3
+/4307	deletion hets	1.8×10^4	2.1×10^3	11.3
4307/+		6.5×10^3	1.2×10^3	18.9
+/161	point mutant hets	4.6×10^4	2.2×10^4	48.0
161/+		3.2×10^3	1.7×10^3	53.6

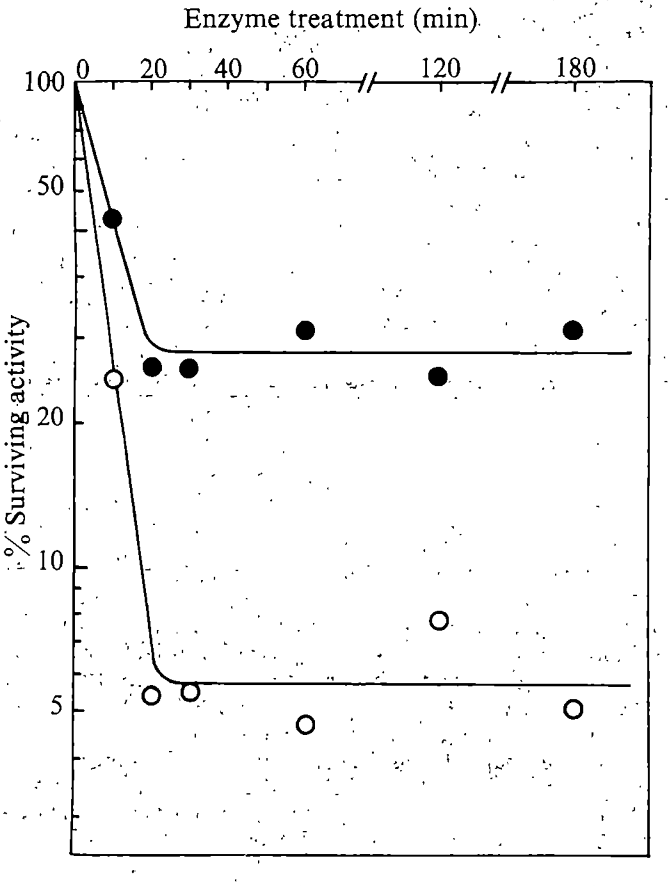
All types of DNA incubated for 15 min at 37 °C with 1/20 dilution of enzyme solution.

inactivation. Heteroduplexes containing the deletion 4307 are inactivated extremely rapidly in comparison with those containing a single base-pair mismatch (Table 2), suggesting a correlation between the distortion in the DNA and enzyme inactivation.

The kinetics of nuclease activity on homoduplex and heteroduplex DNA were followed by mixing DNA and enzyme at zero time. At appropriate intervals thereafter, samples were removed, transferred to tubes containing competent cells and incubated for 30 min at 37 °C to allow for maximum DNA uptake. The results of one such experiment are given in Fig. 1. The initial rate of heteroduplex inactivation was more rapid than with the homoduplex, but both types of DNA reached maximum, but different, inactivation levels after 15–20 min with DNase I. A proportion of the DNA molecules, yielding about 30 % infective centres (homoduplex) and about 5 % infective centres (heteroduplex), seemed to become resistant to the nuclease (see also Table 1).

The plateau in Fig. 1 could be due to a loss of enzyme activity after 15 min incubation or to a fraction of DNA molecules which is resistant to inactivation by the enzyme. The first alternative was excluded by adding fresh enzyme to three types of DNA that had already been incubated with the enzyme for 15 min. No further decrease in biological activity was observed (Fig. 2*a*). The second alternative was supported by the following experiment. We first incubated homoduplex DNA (161/161) and *Ustilago* DNase for 25 min, then added wild-type native SPP1 DNA and subsequently followed the kinetics of inactivation of both 161 DNA and wild type DNA. In SPP1 the wild type plaques, which form a halo, are easily distinguishable from the 161 plaques, which are haloless. This enabled us to follow the kinetics of inactivation of both mutant and wild type DNA simultaneously. Figure 2*b* shows that active nuclease molecules were present in the enzyme–DNA mixture that could not degrade the initial substrate any further, but efficiently attacked any new substrate that was presented.

Fig. 1 Kinetics of enzyme inactivation of homoduplex (●) and heteroduplex (○) DNA.



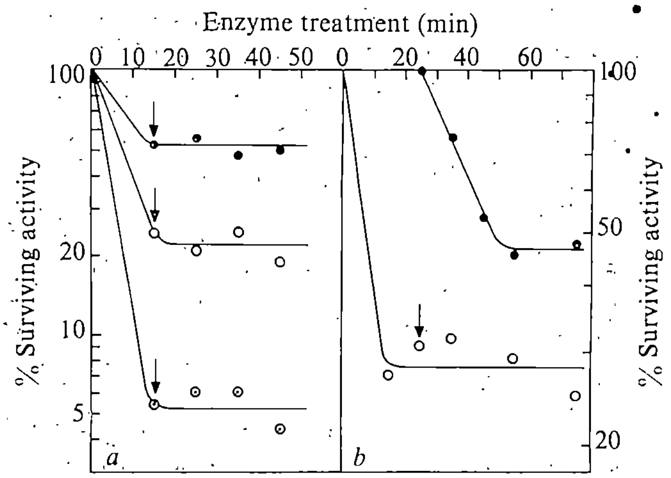


Fig. 2 *a*, Inactivation of native (●), homoduplex (○) and heteroduplex (○) DNA. The arrows indicate time of addition of further enzyme (5 µl 1/10 dilution). *b*, Initial inactivation of homoduplex (○) DNA, followed by inactivation of native DNA (●) added after 25 min.

As we have mentioned, DNase I requires free ends of DNA for activity[16]. It is possible that after binding to an end the enzyme diffuses along the DNA, introducing nicks at points where normal Watson–Crick base pairing is disturbed. We suggest that the preparation of DNase I we used contained a small fraction of defective or partially denatured molecules which bind to the ends of double-stranded DNA, but remain attached there. These would then prevent normal enzyme gaining access to the internal regions of the DNA. If our interpretation is correct, then the difference in the resistant fraction of native (~ 50 %) and heteroduplex DNA (~ 5 %) implies that normal enzyme molecules often monitor the former without introducing a nick. Alternatively, a population of DNA molecules which all contain a single-strand nick could have a residual biological activity of ~5 %. We do not yet have enough data to distinguish between the two possibilities.

We have previously compared the relative sensitivity of irradiated and unirradiated native T7 DNA to the *Ustilago* nuclease. Alkaline sucrose sedimentation patterns show that the enzyme prefers a substrate containing dimers[14]. In the SPP1 transfection system one can irradiate either heavy or light strands before hybridisation (the H strand has 20 % more pyrimidines than the L strand). As Fig. 3 shows, such hybrids when incubated with DNase I show a greater loss of biological activity than the untreated hybrids. In addition, we see a greater degree of inactivation when the irradiated strand is heavy.

Specificity and biological role of the enzyme

It has been shown that a nuclease isolated from *Neurospora* recognises and degrades single-stranded regions in deletion–wild type heteroduplexes[21]. Recently a similar nuclease (S1), isolated from *Aspergillus*[18], was also shown to be specific for

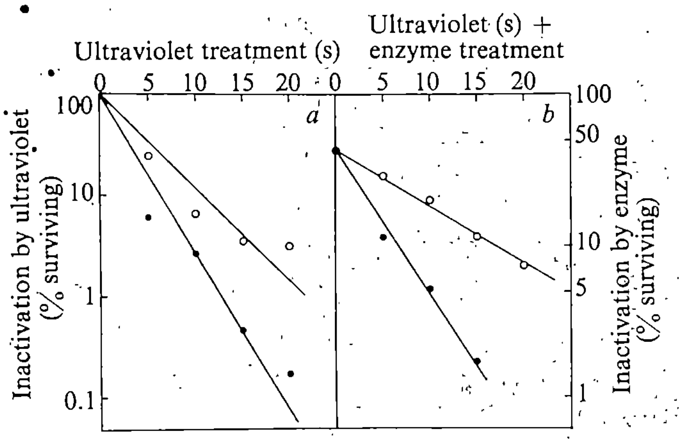


Fig. 3 Inactivation of DNA by ultraviolet light followed by DNase treatment. Single strands, either heavy (●) or light (○), were exposed to ultraviolet light (Sterisol, Hanau lamp, emitting 1.1 J m^{-2} s^{-1} at 30 cm). After hybridisation, DNA was treated for 15 min with DNase (as described in Table 1). *a*, Inactivation by ultraviolet without enzyme treatment; *b*, additional inactivation of the same substrates by a constant enzyme treatment.

mismatch-containing substrates[22]. The involvement of these enzymes in genetic processes is unknown because of the lack of mutants deficient in the enzymes. In the case of *U. maydis*, however, there is clear evidence that mutant strains which have a reduced amount of the enzyme in crude extracts have a barely detectable level of gene conversion[13,14].

The results we have obtained demonstrate that DNA containing a single purine–pyrimidine mismatch, which presumably produces a very minor distortion in DNA, is a preferred substrate to an identical one without the mismatch. A deletion–wild type heteroduplex is even more sensitive to the enzyme, possibly due to a greater distortion in the DNA. It is perhaps significant that addition or deletion mutants of *Ascobolus immersus* show gene conversion much more frequently than do base substitution mutants[12]. With the latter, postmeiotic segregation often occurs, suggesting that the correction enzyme is not very efficient at recognising these minor distortions in hybrid DNA.

The discovery of an enzyme which can recognise DNA containing a single mismatch provides strong evidence for the hypothesis that gene conversion is due to the correction of mismatched bases in heteroduplex DNA *in vivo*. As in ultraviolet repair by excision of pyrimidine dimers, it is likely that the overall correction process involves a mismatch-provoked degradation and resynthesis of a short region of single-stranded DNA[1,23,24]. DNase I may be involved only in the first step of this process. In the absence of information concerning the chemical nature of the degradation products of the enzyme, we cannot say anything about the actual event which leads to biological inactivation in our system.

The knowledge that enzymes exist which are specifically involved in mismatch repair leads one to consider the biological function or significance of such a correction mechanism. It is believed that incorrect bases may be removed at the replication fork by the editing function of DNA polymerases[25]. If this editing fails, however, and an incorrect base is inserted into DNA, correction enzymes such as DNase I could come into operation. Random repair provides no biological advantage over the segregation of mutant and wild-type strands after replication. One would therefore need to assume non-random repair leading to the excision of the newly synthesised 'wrong' strand. This could conceivably happen at the replication fork, where discontinuous synthesis would provide the necessary ends required for nuclease activity. If these nucleases are involved in general repair, it would be expected that mutants lacking such repair would have a mutator phenotype. Evidence suggests that *Pneumococcus* strains lacking the capability for heteroduplex repair have an abnormally high rate of spontaneous mutation[26]. An alternative or additional function of a correction enzyme could be the recognition and removal of damaged or abnormal bases in DNA, such as uracil, derived from the spontaneous deamination of cytosine[27]. Enzymes which recognise base pairs such as uracil–guanine may also detect other abnormal base-pair combinations.

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letters to nature

New non-stellar BL Lacertae objects OY091 and PKS2335+03

DURING the past year, opinions on the nature of the BL Lacertae objects have narrowed to the association of elliptical galaxies with non-thermal sources which display continuous optical spectra. The study of the objects B2 1101+38 (Markarian 421) and B2 1652+39 (Markarian 501) indicates that they could be members of a sequence of elliptical galaxies ordered by the relative brightness of the BL Lac objects located in their nuclei[1]. On the other hand, the proximity of B2 1215+30, ON231, PKS1205−008 and PKS0048−097 to galaxies or nebulosities, has prompted the suggestion that, at least these four BL Lac objects have been ejected from the nuclei of their companion galaxies (J. J. Condon and D. L. Jauncey, unpublished). Furthermore, an analysis of the optical energy distribution[2] of

Table 1 Details of exposure

Exposure (min)	Filter	Emulsion
8	RG-2	103a-D
15	6,000–7,200Å (HBW)	103a-D
30	RG-2	IIIa-J

several BL Lac objects indicates that the decline of their optical spectra toward the ultraviolet becomes steeper because of the contribution of underlying elliptical galaxies. Typical examples are Markarian 501, AP Librae and BL Lac itself. In the case of AP Librae, the nebulosity was discovered with deep image tube photographs[3].

We undertook a search for nebulosities underlying the BL Lac objects 4C14.60, OT081, OY091 and PKS2335+03 and obtained positive results in the last two cases. These four objects appear stellar on glass copies of the National Geographic–Palomar Observatory Sky Survey. The selection of these objects was based on the steep decline of their spectra towards the ultraviolet[2]. Our observations were made with a 140-mm ITT image tube camera with a red-extended S-20 spectral response. The camera was used at the Cassegrain focus of the Steward Observatory 2.25-m telescope to obtain a 23′ field with a 10″ mm^{-1} scale. On the nights of August 2 and 3, 1975, we made three exposures of each of these four objects (see Table 1).

The objects 4C14.60 and OT081 remain stellar in appearance to the limit of our plates ($V \sim 22.5$ mag); however, both OY091 and PKS2335+03 exhibit non-stellar images on all of our plates. OY091, shown in Fig. 1, has a very soft image, with surrounding nebulosity highly reminiscent of that seen on deep plates of AP Librae[3]. Transmission scans in one dimension across the disk of OY091 clearly confirm the non-stellar image. The object PKS2335+03, shown in Fig. 2, is perhaps more interesting because the observed structure is clearly extended to the east, although there is also some fainter structure to the west. The distinct eastward extension resembles a jet or an extremely close companion.

The fields around OY091, PKS2335+03 and 4C14.60 contain many faint galaxies, suggesting a possible membership of these three objects with distant clusters of galaxies. In this respect OY091, PKS2335+03 and 4C14.60 are similar to PKS0548−322, which has been found to be projected on to a cluster of galaxies[4]. No evidence for a cluster of galaxies is observed on our plates of OT081; however, the low galactic latitude ($b^{II}=17.7°$) of this object, precludes a definitive statement. The association of BL Lac objects with clusters of galaxies is a matter which deserves further study.

Fig. 1 This reproduction of the field of OY091 is from a 30-min exposure with an RG-2 filter on a IIIa-J emulsion. North is at the top; east is to the left.

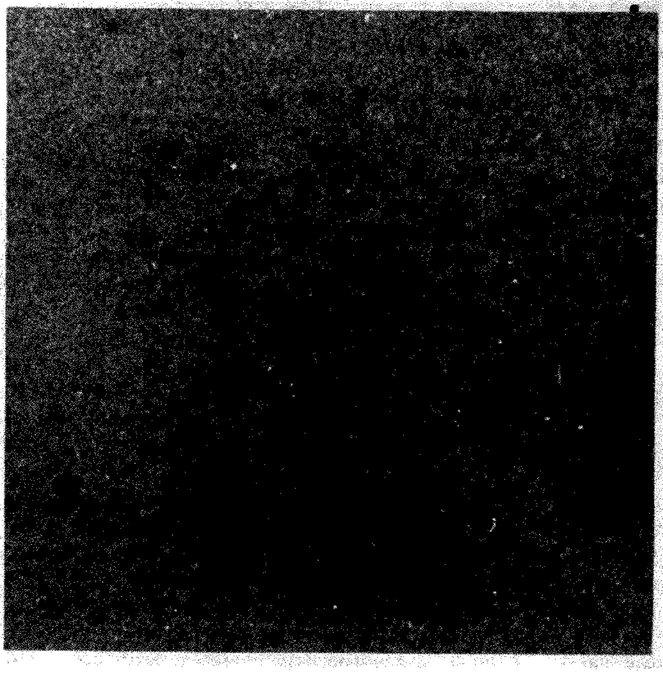

Fig. 2 PKS2335+03 is shown with the nebulous extension to the east in this reproduction of an 8-min exposure with an RG-2 filter on a 103a-D emulsion. Orientation as in Fig. 1.

The morphological classification of BL Lac objects suggests three possible groups: (1) objects located in galaxies, (2) objects associated with nearby galaxies and (3) isolated objects. Since the majority of the BL Lac objects seem to be somehow related to galaxies, it seems of interest to ask whether isolated BL Lac objects really exist, or will they eventually be found to be associated with galaxies? Because the ability to detect faint nebulosity, using photographic techniques, is a strong function of the distance to the object, it would be useful to find other observational parameters to detect associated galaxies. When a BL Lac object is located in a galaxy, the steepness of the optical energy distribution seems to be a useful parameter. The detection of underlying nebulosity in OY091 and PKS2335+03, two objects with spectral indices ($d \log F_\nu / d \log \nu$) of −2.7 and −2.3 respectively[2], tends to confirm this suggestion.

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Cosmic rays and interstellar tunnels

MEASUREMENTS have been made[1] of the isotopic abundances of Be in cosmic rays, which have led to the conclusion that the typical cosmic ray lifetime in the galaxy is $\sim 2 \times 10^7$ yr. Furthermore, measurements[2] of the cosmic ray electron energy spectrum in the range 6–100 GeV, have shown a bend in the spectrum which can be attributed to inverse Compton and synchrotron losses. The position of the bend is indicative of the cosmic ray electron lifetime, and an age of 1.2×10^7 yr is derived.

These two values are significantly longer than the widely quoted value of $\sim 3 \times 10^6$ yr. This latter value is consistent with the average column density of material traversed by cosmic rays, ~ 5 g cm^{-2} (as determined by measurements of products of spallation reactions), and the usually assumed average density of matter in the galactic disk (~ 1 H atom cm^{-3}).

Measurements of the interstellar density indeed show[3] $\rho_{\mathrm{is}} \sim 1$. The newly determined cosmic ray lifetime, however, implies that the average density through which the particles have travelled is ~ 0.2 atom cm^{-3}. This value is an upper limit to the average density encountered by cosmic rays in the galaxy at large, since there is evidence indicating that cosmic rays traverse a large fraction of the above mentioned column density at the particle sources[4]. It is therefore apparent that cosmic rays must spend $\sim 80\%$ of their lifetimes in regions of the galaxy where the density is significantly less than the average value found in the disk.

For this reason, the existence of a cosmic ray "halo" has been postulated[1,5]. This halo is a low-matter density region of cosmic ray confinement which surrounds the galactic disk.

Webster[6], however, analysed data concerning the radio halo of the galaxy, and found that the idea of static confinement in the halo is inconsistent with the observations. Dynamic confinement such as that proposed by Jokipii[5] may explain the low mean density traversed by cosmic rays.

An alternative explanation is proposed here. The low mean density traversed may be indicative of the presence of hot, low density tunnels in the interstellar medium such as those proposed by Cox and Smith[7]. Cosmic rays produced in supernovae or in supernova remnants are preferentially injected into these tunnels. The tunnels act as cosmic ray traps in which the density is low ($\sim 10^{-3}$ atom cm^{-3}), thus reducing the mean density encountered over the particle lifetime.

Many of the theories of the origin of cosmic rays involve particle acceleration by supernovae explosions, by the resultant pulsar, or in the supernova remnants. Analysis of cosmic ray abundances[8] supports the view that cosmic rays are accelerated, at least partially, in supernova remnants (compare with ref. 9 and J. R. Jokipii, personal communication). Indeed, γ-ray observations of the Vela supernova remnant[10] show that it is likely that supernovae or supernova remnants both produce and confine enough cosmic rays to explain the observed galactic cosmic ray density.

Cox and Smith[7] estimate that a fraction, f, of the volume of the interstellar medium is occupied by hot, tenuous tunnels caused by linked chains of supernova remnants, where $0.1 \lesssim f \lesssim 0.5$. Since typical remnants expand to a radius of ~ 40 pc before they start to disappear, they have a probability, P, of intersection with an existing tunnel, such that $0.6 \lesssim P \lesssim 1$. When the shock front of a new remnant encounters an existing tunnel, the shock is preferentially driven along the tunnel. Thus the remnant material which contains the cosmic rays produced by the supernova (Higdon and Lingenfelter's[10] data indicate that cosmic rays are confined up to a remnant radius of $\lesssim 30$ pc) is injected into the tunnel system.

As was pointed out previously[7], the tunnel systems are excellent cosmic ray traps, since they are regions of low magnetic field. The tunnels are intertwined about the large scale flux tubes of the galactic magnetic field. Particles injected into the system are therefore internally reflected by the high magnetic fields in the tunnel walls. The particles can escape only by slowly diffusing on to the galactic field lines.

The most likely mechanism of particle escape from the tunnel is resonant pitch-angle scattering with turbulent fluctuations in the interstellar field. The typical (~ 3 GeV) cosmic ray particle has a gyro radius $\sim 10^{12}$–10^{13} cm in the galactic field ($\sim 3 \times 10^{-6}$ gauss). Unfortunately, we have no observations of the scale size of the turbulence in this regime, and this makes it difficult to estimate the time scale of escape. It is, however, interesting to note that this mechanism will, if operative, give an energy dependence to the cosmic ray spallation products. This is because particles with different gyro radii (that is, different energies) will scatter most effectively off correspondingly different sized fluctuations, and the probability of encountering a given size fluctuation is generally a function of the size of that particular turbulent element. This effect produces a variation in the fraction of the lifetime of the particles which is spent in the high density regions of the disk, and thus a variation in the spallation products. Energy dependence of spallation products is indeed observed[11], although an alternative explanation has been suggested[9].

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Measurements of the far-field resonance cone for whistler mode waves in a magnetoplasma

USING a large volume laboratory magnetoplasma the wave fields and resonance cone of the right hand polarised (whistler mode) wave have been mapped at distances of up to 10 whistler wavelengths from a transmitting antenna. The results are in good agreement with theory and suggest that energy is convected away from the antenna along the surface of the resonance cone. Considerable attention has been paid to the generation of waves by a point source in a magnetoplasma, and theoretical analyses[1–5] have shown that for certain combinations of the antenna operating frequency ω, the electron gyrofrequency ω_c, and the plasma frequency ω_p, the fields become singular along a cone whose axis is parallel to B_0, the steady magnetic field. By examining the refractive index surfaces it can be seen that the group velocity of the wave is zero when the ray vector is parallel to the resonance cone of half angle θ (when the wave normal is at an angle $\pi/2-\theta$ to B_0). We restrict ourselves here to a discussion of the radiation pattern for frequencies $\omega<\omega_c\ll\omega_p$. Waves launched by the antenna then propagate in the right hand polarised whistler mode and the angle of the resonance cone is approximately given by $\theta=\sin^{-1}(\omega/\omega_c)$.

This phenomenon is of particular interest because it defines a narrow region of space in a magnetoplasma where the group velocity is very small. The question then arises as to whether energy is, in fact, transferred along the resonance cone or is merely a near-field phenomenon. If the cone exists in the far field it would have a significant effect on the propagation characteristics of the wave and the impedance of the transmitting antenna. This information would be needed with any mother–daughter spacecraft experiment where the dispersion of a wave travelling between two spacecraft is interpreted in terms of the local plasma parameters. It is also needed in laboratory experiments on controlled thermonuclear reactions where radio frequency energy is used to heat the charged particles to high temperatures.

The behaviour of the cone angle as a function of ω, ω_0 and ω_p has been studied experimentally in the near field[5,6] and is reasonably well understood. The far field of the cone and wave fields have been studied theoretically[7] but little other work has been done on this part of the problem. In particular, the shape of the wave fronts in the cone and the propagation of energy along the cone surface are problems yet to be solved.

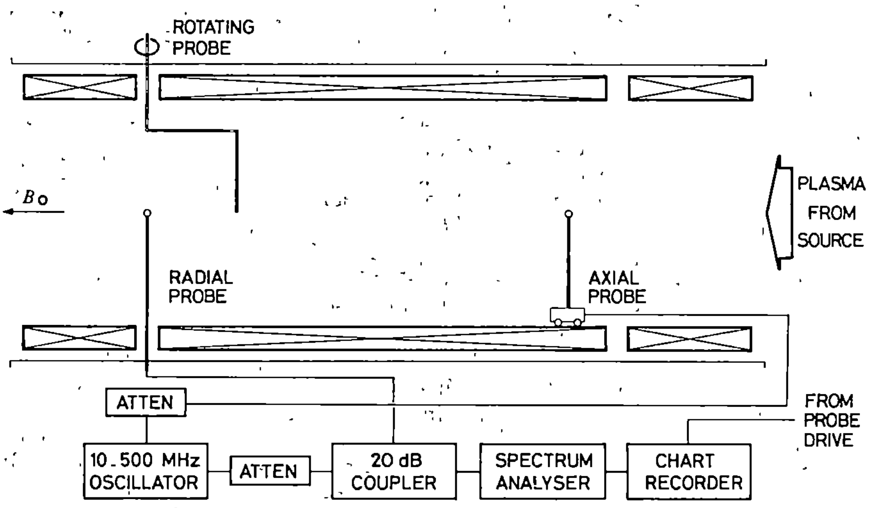
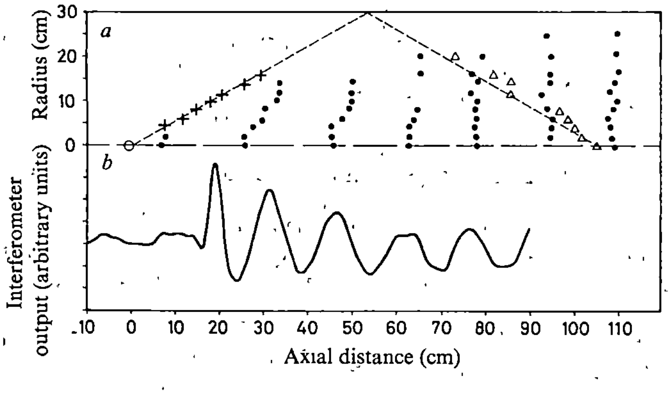


Fig. 1 Experimental arrangement of the solenoids and probes.

Using small, movable probes in a large, laboratory magnetoplasma, I mapped the radiation field of an antenna over a distance of up to 10 wavelengths of the whistler wave. For convenience, a frequency of about half that of ω_0 was used since it was thought that the focusing effect predicted by Wang and Bell[7] may be observable, although they investigated the very far-field radiation pattern at a distance of approximately 6,000 wavelengths.

Fig. 2 *a* Results derived from the interferometer measurements: crosses, positions of the resonance cone; triangles, positions of the cone after reflection from the wall of the vessel; dots, surfaces of constant phase of the whistler wave (each set is 2π in phase apart). *b*, An interferometer trace taken at a radial distance of 10 cm.



The experiment was performed in a large volume magnetoplasma[8], 60 cm in diameter and approximately 150 cm long. An axial magnetic field was provided by three solenoids giving an axial uniformity of ±0.6% over 120 cm and 0.01% uniformity over the central 10 cm. Fields of up to 300 gauss can be obtained (see Fig. 1).

A plasma was formed in a glass tube 15 cm in diameter at one end of the vacuum chamber by radio frequency ionisation at 80 MHz. The oscillator produced about 1 kW of radio frequency power which was coupled into the plasma inductively. The coupling between the source axial magnetic field and the main field allowed the plasma to drift into the experimental volume, producing a radial gradient in density, which was approximately Gaussian.

Langmuir probe measurements gave the electron temperature as 2.6 eV, in good agreement with results derived from measurements of the interference structure of the resonance cone[9]. For this experiment the magnetic field was set at 64 gauss, with an Argon gas pressure of 4×10^{-4} mmHg giving a collision frequency—essentially caused by electron—neutral collisions—of about 0.0005 ω_0. Any dissipative effects would, therefore, be of a collisionless nature.

By measuring the whistler wavelengths over two decades in frequency a graphical plot of the dispersion was obtained. This was compared with theoretical curves using ω_p as a fitted parameter, and yielded an average value for the electron density of 4×10^{10} cm^{-3}.

The transmitting antenna had a loop 1 cm in diameter at the end of a coaxial probe 3 mm in diameter which could be moved along the length of the vessel. Receiving antennae were similar loops or small electric probes with an exposed surface 1 mm in diameter and 4 mm long. The wave fields were mapped by setting the receiver at a certain radial position and moving the transmitter axially.

Since it was found that the resonance cone itself was reflected from the metal walls of the vessel, this method of moving the transmitter on the axis maintained symmetry and allowed the position of the reflected cone field to be determined easily.

A simple interferometer was constructed using the received signal as one arm and a direct signal from the oscillator driving the transmitting antenna as the other arm. The two signals were mixed and displayed on a spectrum analyser set to zero scan (that is, operating as a narrow band superheterodyne receiver) and a permanent copy was made on an *x*–*y* chart recorder.

Figure 2*a* shows results taken from interferometer traces using a loop receiving antenna for a frequency of 150 MHz

($0.47\ \omega_c$). The positions of the reflected cone, shown by the triangles, could be determined from the traces as a rapid change of phase which could be distinguished easily from the whistler wave fields. For this particular wave frequency the theoretical cone angle is 28°, in good agreement with the experimentally measured value of $28 \pm 1°$.

At higher magnetic fields, B_0, we have measured the resonance cone field at distances greater than 10 wavelengths from the transmitter, where the wavelength referred to is that for a longitudinally propagating whistler wave. This would seem to suggest that energy does in fact propagate along the cone surface away from the transmitter in contrast to the assumption of Wang and Bell[7] that the radiation intensity near the cone has little physical significance.

Within the cone, the wavefront can be seen to be curved. By examining the refractive index surface and constructing wavefronts, a theoretical wave front can be determined, and that is in good agreement with our measured results. Further away from the cone, the effects of the cylindrical geometry must be taken into account. As the diameter of the vessel is much larger than the wavelength, the dispersion of the waves is little changed but the shape of the wavefront is planar in cylindrical geometry and the gradual change from curved to planar can be seen in the figure.

A maximum variation of about 20% in wavelength along the axis shows that the axial gradient in density caused by the drifting plasma is probably of the same order of magnitude. The radial gradient in density does not seem to have any significant role in the formation of the wavefronts.

An interferometer trace using a loop receiving antenna taken at a radial distance of 10 cm is shown in Fig. 2*b*. The first maximum corresponds to the resonance cone and following maxima are caused by the wavefields. The nonzero signal to the left of the resonance cone is caused by the interferometer, the wave field outside the cone being essentially zero. By moving a probe radially, the wave fields were found to be greatest along the axis, decreasing gradually as the resonance cone is approached. Although we cannot regard this as a measurement of the focusing effect suggested by Wang and Bell[7], it is quite possible that after many more wavelengths the fields would be concentrated about the axis.

The increasing area of the wavefront as it moves away from the transmitter can be seen as a gradual decrease in the wave amplitude.

I am at present mapping the radiation fields and, in particular, the relative amplitudes phases and directions of E and H for a wide range of frequencies below the electron gyrofrequency, especially below $\omega = 0.2\omega_c$, where the ray direction can theoretically be outside the resonance cone. These results will be presented elsewhere.

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Subducted greywacke in the Olympic Mountains, USA—implications for the origin of Archaean sodic gneisses

IN Archaean greenstone belts the earliest granitic rocks are sodic or quartz dioritic gneisses and migmatites that surround and diapirically intrude the greenstones. The granitic rocks have been considered as the sialic basement on which the greenstones were deposited[1,2] and as sialic underplatings of a primordial greenstone crust[3–5]. But the structural relationships between greenstones and greywackes in the Olympic Mountains of Washington State, USA, suggest that the Archaean gneisses may have formed by subduction of greywacke suites and associated volcanic rocks beneath nearly coeval oceanic crust.

If the Archaean granitic rocks are primary sialic basement[1,2] the sodic gneisses must be older than, and beneath, the oldest Archaean greenstones. This possibility is attractive, since gneiss domes in younger terrains are generally regarded as remobilised basement rocks, and the sodic migmatites are compositionally similar to the diapiric plutons[4]. Contacts between the oldest greenstones and sodic granitic rocks are, however, invariably tectonic or intrusive. No unequivocal examples are known where the oldest greenstones lie unconformably over granitic basement[4–7]. Unconformities do exist within the Archaean, but the basal layers comprise sedimentary rocks, mostly conglomerates, resting on granitic rocks[6–8]. Further, 'granitic' clasts in Precambrian volcanic–sedimentary belts do not occur in the basal greenstones, and clasts higher up in the sequences may have been derived from nearly contemporaneous volcanic and hypabyssal rocks rather than from a pre-existing granitic basement[9].

The Archaean gneisses are, admittedly, of higher metamorphic grade and more highly deformed than the adjacent greenstone belts, but these criteria cannot be used reliably to establish the relative ages of suites that are tectonically juxtaposed. Within the Barberton greenstone belt of South Africa, the Swaziland Supergroup has been dated[4], using Rb/Sr techniques at 3,355 Myr and the surrounding Ancient Gneiss Complex[4] at 3,395–3,138 Myr. This coeval relationship holds for several Archaean greenstone and gneiss terrains[5].

The sodic granitic rocks have also been considered to be sialic underplating (without any supracrustal manifestations) of the primordial greenstone crust[2,4,5]. The granitic migmatic terrains, however, contain relict metasedimentary or metavolcanic features, such as banded iron formations, amphibolites, and quartzites[1,4–6]. If the tonalitic bodies within the migmatitic complex adjacent to the Barberton Greenstone Belt were anatectic additions to the gneisses, the resultant increase in volume should have deformed the neighbouring greenstone belt[4]. Moreover, the anatectic mechanisms necessary to generate vast amounts of sodic granitic material from oceanic crust or the mantle are somewhat difficult to envisage[1,3].

The Olympic Peninsula comprises two almost coeval lithological assemblages (Fig. 1). The mountain core is composed of Eocene–Miocene slate and greywacke with minor lenses of basaltic rocks. These core rocks are strongly folded and faulted and are metamorphosed in the prehnite–pumpellyite to lower greenschist facies[10].

Flanking the mountain core on the north, east, and south is a discontinuous belt of early (?) and middle Eocene marine basaltic rocks, which are succeeded by an Upper Eocene to Miocene marine sedimentary sequence. Although folded and faulted, this succession is generally stratigraphically continuous, and only modestly deformed compared with the core rocks The basaltic rocks of the

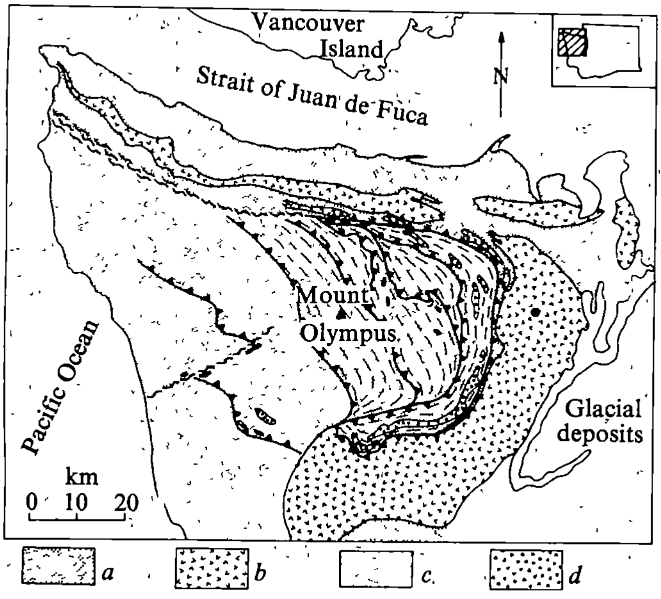

Fig. 1 Structural sketch map of the Olympic Peninsula, Washington (after Tabor[10]). *a*, *b*, Peripheral rocks (orderly, moderately deformed and partially metamorphosed): *a*, Eocene to Miocene sedimentary rocks; *b*, early (?) and middle Eocene volcanic rocks (basalt and greenstone). *c*, *d*, Core rocks (intensely deformed, faulted, and partially metamorphosed): *c*, Eocene to Miocene sedimentary rocks (dashed where penetratively deformed); *d*, Eocene and younger (?) volcanic rocks (basalts and greenstones). *e*, Contact; *f*, thrust fault; *g*, shear zone.

peripheral belt are dominantly pillowed and massive diabasic oceanic tholeiites[11,12], but about 20% are alkalic basalt[12]. An intrusive complex of diabase, gabbro, and porphyritic basalt occurs in the thickest portion of the basalts[12].

Chemically, most of the basalts correlate well with oceanic-ridge tholeiites, particularly in their TiO–FeO/MgO distribution and in rare earth elements, although some of the upper flows show trends typical of oceanic-island tholeiites[12]. Most of the volcanic pile is apparently a slice of upper oceanic crust, with the small volume of alkalic rocks representing the last phases of volcanism[12].

The volcanic rocks have been statically metamorphosed from zeolite facies to prehnite–pumpellyite and, locally, greenschist facies. Many of the flows "might well be called greenstones"[13].

The basalts crop out in an arcuate pattern starting on the north-western tip of the peninsula and extending more than 220 km east and south. Their thickness varies from a few tens of metres in the north-west to a maximum of 15 km on the east[11]. Regionally, these rocks are part of an early (?) to middle Eocene assemblage of marine basaltic rocks that extends from Vancouver Island southwards to the Klamath Mountains, a distance exceeding 600 km.

Structural and palaeontological relationships indicate that the greywackes and slates of the core are of the same age or younger than the basalts and that the core rocks were inserted beneath the peripheral basalts along a thrust mappable over a distance exceeding 120 km[11,14]. Regional gravity data[15] from the eastern portion of the Olympic Peninsula suggest that the basalts are a slab about 15 km thick, dipping 45 degrees eastwards.

The extreme deformation and thrusting of the core rocks under the peripheral basaltic rocks was probably the result of pre-Pliocene subduction. Subduction is indicated by the ages and patterns of linear magnetic anomalies in the adjacent north-eastern Pacific Ocean[16].

The few published analyses of K_2O–Na_2O ratios in Olympic core rocks[17,18] and the K_2O/Na_2O plots of Tertiary greywackes, Archaean greywackes, and Archaean sodic granitic gneisses[5] yield results of about 0.5. Thus, isochemically metamorphosed Tertiary or Archaean greywackes could yield rocks with the same K_2O–Na_2O ratio as Archaean sodic granitic rocks. This suggestion is an extension of the inference[19] that greywackes may be transformed isochemically in to banded albite schists and gneisses. Mineralogically, chemically, and in some cases even texturally, the sodic migmatites are very similar to the sodic diapirs that intrude the Barberton Greenstone belt[4]. The presence of basaltic rocks within the greywackes of the core of the Olympic Mountains and of amphibolites in the Precambrian migmatitic terrains is especially interesting in view of Glickson's comment that "Tonalites and granodiorites intruded into Archaean systems commonly abound in basic xenoliths . . .; such altered xenoliths represent remnants of basic rocks intruded by the acid melts thus pointing to an origin of the melts from below a basic crust."[3].

We concur with Anhaeusser[20] that plate tectonic models for Archaean time are viable, and suggest that the Archaean sodic granitic rocks may have been supracrustal rocks that were subducted beneath coeval oceanic greenstones. This hypothesis avoids the problems inherent in postulating a primordial sialic crust, the requirement of large scale anatectic granitic underplating of the greenstones, and the extreme lateral variations in geothermal gradients otherwise needed[5] to account for adjacent Archaean belts of vastly different metamorphic grades.

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Plate tectonic significance of repeated tectonic trends in eastern Canada

THE existence of generally parallel, major tectonic structures of different ages has been long recognised. In Eurasia, for instance, the Alpine Orogenic Belt is paralleled by the Hercynian–Variscan Orogenic Belt, and in southern New Brunswick there is a general parallelism between the Alleghenian (Variscan) and Acadian fold belts[1]. In Newfoundland[2] the Acadian Fold Belt runs generally parallel to the older, late Cambrian–earliest Ordovician (Grampian) Fold Belt in the west and a pre-middle Ordovician fold belt in the east. Further, the Appalachian–

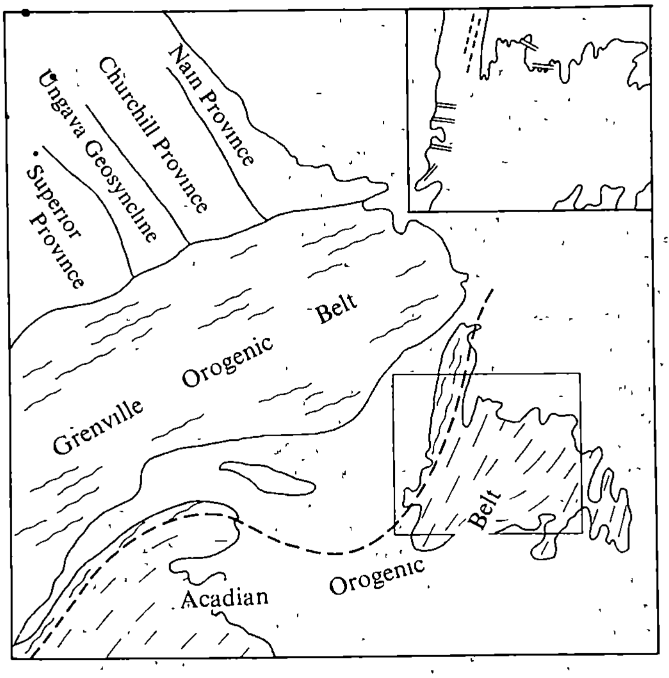

Fig. 1 Tectonic features of eastern Canada Inset (showing boxed area): dashed lines, dykes cutting the Grenville Province; solid double lines, sheeted dykes.

Caledonian Belt as a whole in north-eastern Canada runs parallel to the older (1,000 Myr BP) Grenvillian Orogenic Belt for at least 1,000 miles. These are clearly indications of continental accretion (Fig 1).

The Precambrian and Phanerozoic Belts which run parallel to the North American seaboard of the north Atlantic Ocean are terminated transversely by the Labrador Sea. Thus, in terms of pre-Mesozoic fit, the correlation of the Grenville Formation[3] with the British Isles is uncertain. The Caledonian and northern Appalachian Orogenic Belts, in spite of their apparent continuity from Newfoundland to the UK, are not of identical ages. The Caledonian Belt generally reached a climactic episode during end-Silurian-early Devonian times, whereas the northern Appalachian Belt was generally deformed during the mid-Devonian. An important factor in the interpretation of the cutoff against the Labrador Sea is the nature of the ophiolite complexes in Newfoundland. Dewey and Bird[4] have drawn attention to the significance of Newfoundland ophiolites as portions of old (early Ordovician and older) oceanic plate. Later work[5-8] resulted in a wider recognition that these ophiolite complexes could be remnants of Ordovician or older small oceans, with an internal structure of oceanic lithosphere, including basaltic pillow lavas, sheeted dykes, gabbros and upper mantle peridotites.

Dewey and Bird's model envisaged that the deformation of the sedimentary prism marginal to the continent was contemporaneous with the subduction of the oceanic crust. In this respect the oceanic crust of the small oceans in Newfoundland needs further study, as it includes some unexplained features. First, the fragments of oceanic crust lie tectonically over[9] or, at least, almost against the sialic crust[5]. Second, their internal structure cannot be related to the deformed sialic rocks since the sheeted dyke complexes are at a high angle to the trend of orogenic deformations (Fig 1, inset) The direction of the initial spreading of the preserved oceanic crust is thus not parallel to the direction of final closing (Fig 1, inset) of the ocean as a whole.

The relationship between the ophiolites and the adjacent continental rocks along the northern coast of Newfoundland can be interpreted in terms of obduction, rather than subduction. The trend of the dykes in the sheeted complexes suggests that the small ocean whose strike is indicated by the sheeted dyke complex, had a trend radically different from the larger ocean to which the Appalachian Orogenic Belt of Newfoundland was marginal. This larger ocean is often referred to as the proto-Atlantic[9]. It is significant that the trend of the Ordovician(?) sheeted dykes is sub-parallel to the present magnetic lineations of the Labrador Sea[10,11]. It seems reasonable to suggest that the small Ordovician ocean had the same relation to the proto-Atlantic as the present Labrador Sea has to the modern Atlantic Ocean. If that is so, then the preservation of parts of the small ocean (proto-Labrador Sea) could be explained by the hypothesis that, when the proto-Atlantic Ocean closed, a small, oceanic, proto-Labrador Sea became completely land locked. Similarly, were the modern Atlantic Ocean to close, one would expect the oceanic crust of the present Labrador Sea to be preserved at a high tectonic level and to obduct on to the adjacent Greenland–Canadian mainland. It would not subduct because it would be situated inside the subducting margin of the closing Atlantic Ocean.

The hypothesis has further implications, in so far as it suggests that a triple junction of the type that now exists at the meeting point of the North Atlantic Ocean, the north-eastern Atlantic Ocean and the Labrador Sea must have existed at approximately the same location during early Palaeozoic times. Thus, the cutoff of the Grenville Belt becomes more intelligible and the time difference between the Caledonian and Acadian orogenies is explicable by regarding the two branches of the orogenic belt as lying on opposing sides of the triple junction. Furthermore, the Labrador branch of the Ungava Geosyncline[12], deformed during the Hudsonian Orogeny, may have represented a margin to a still earlier 'Labrador Ocean' which existed there some 2,000 Myr BP. It is fitting to suggest a longevity of certain tectonic situations in geological time and the existence of lithospheric weaknesses which are repeatedly subject to separation and collision.

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Mesospheric and lower thermospheric wind changes over Canada during the January 1975 stratospheric warming

EVIDENCE indicates that the mesosphere and lower thermosphere may be perturbed when stratospheric temperatures increase rapidly in winter Much of this evidence is indirect, due to limitations inherent in existing techniques of observation, and typically comprises measurements of ionospheric

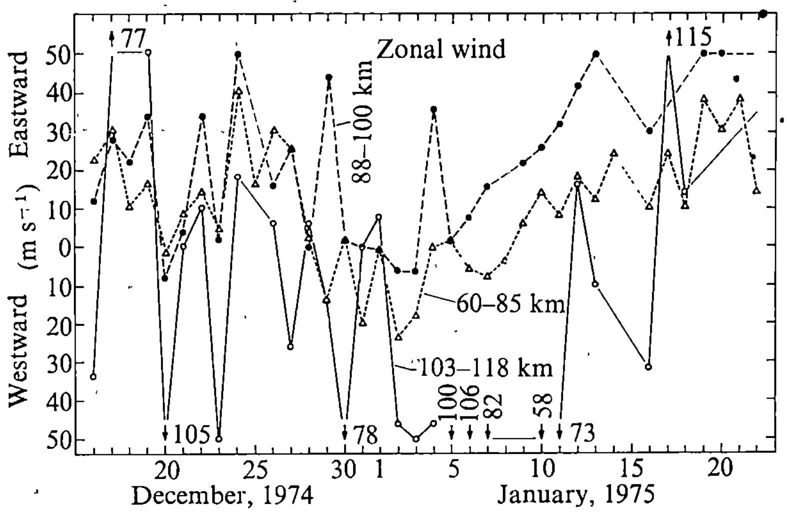

Fig. 1 Zonal component of daily median wind, for one hour around noon, and in the altitude ranges 60–85, 88–100 and > 103 km, at Saskatoon.

conditions, such as absorption in the lower ionosphere or heights of isoelectronic surfaces up to the E-region. Changes in these conditions are found to be significantly correlated with increases in stratospheric temperatures in winter at latitudes above ~40°.

There seem to be at least two related classes of stratospheric temperature changes. The first is an aspect of a travelling planetary wave—changes in the mesospheric electron densities associated with such waves have been observed in both the Southern Hemisphere[1], and Northern Hemisphere[2,3]. The second, which is usually called a stratospheric warming or a major warming, is the reversal, in winter, of the polar vortex to westward flow. It is thought to be initiated by the interaction of a travelling planetary wave with a standing wave[4,5]. Ionospheric changes related to major warmings have been identified in the Northern Hemisphere[6]. In the Southern Hemisphere, the interaction of travelling and standing planetary waves may not occur to the same extent as in the Northern Hemisphere.

Observations of the winds and temperatures of the mesosphere and lower thermosphere during stratospheric warmings are relatively few. Significant correlations between the temperatures at 30 km and the pressures at 55 km for Fort Greely, Alaska, and between temperatures at 30 km and pressures at 70 km for Wallops Island, Virginia[7] have, on occasion, been found. A reversal of zonal flow between 75 and 105 km, from eastward to westward, has also been observed in Alaska by means of the radio meteor technique during the warmings of the winters of 1967–68 and 1970–71 (ref. 8).

In the winter of 1974–75 observations of the winds from 50 to 105 km, were made near Saskatoon (52°N, 107°W) and Ottawa (45°N, 76°W), using the partial radiowave reflection, drift-of-diffraction-pattern technique[9]. Together with meteorological rocket measurements from Churchill (58°N, 94°W), these provide more detailed wind data with better continuity in the vertical direction, than previous experiments provided. The Churchill data (not shown) establish that a major warming was detectable around December 17, when the wind at altitudes between 30 and 40 km began to shift from eastward through southward, to a reversed (or westward) flow, which prevailed on January 9. On those dates, temperatures at altitudes between 30 and 40 km were ~ −60 °C and ~ −30 °C respectively. The flow remained westward until about January 17. For comparison, Fig. 1 shows the behaviour of the zonal component of winds at Saskatoon in three altitude ranges: 60–85, 88–100, and ⩾103 km. (The height at which total reflection of radio waves at 2.2 MHz is observed, occurs in the latter range.)

Five-year mean values (J. B. Gregory and A. H. Manson, unpublished), have established that the zonal component is normally eastwards up to ~ 95 km at this time of year, in the absence of perturbations caused by warnings. Above 95 km, the flow is normally westwards, and a westward component of the thermal wind, corresponding to a warm high-latitude mesosphere, also normally exists above 75 km during this part of the winter at Saskatoon. Figure 1 shows two forms of departure from this. The first is a short term variability, over intervals of 1–4 d, and is greatest above 100 km. This variability is believed to be due to the effect of gravity waves ($\tau \sim$ 60 min) when, because of incomplete data, they are not removed by height and time averaging The second departure, evident between about December 30 and about January 12, is of interest here, since it coincides with the warming observed at Churchill.

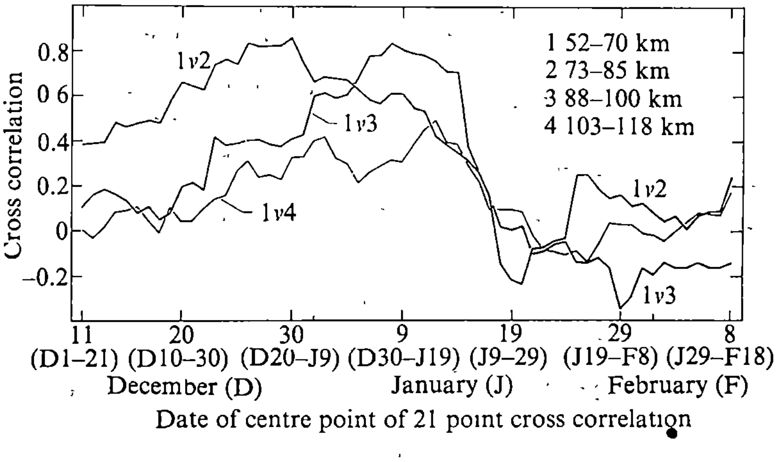

Fig. 2 Cross-correlation coefficients of zonal component of winds at Saskatoon, between four altitude ranges 52–70, 73–85, 88–100 and 103–118 km.

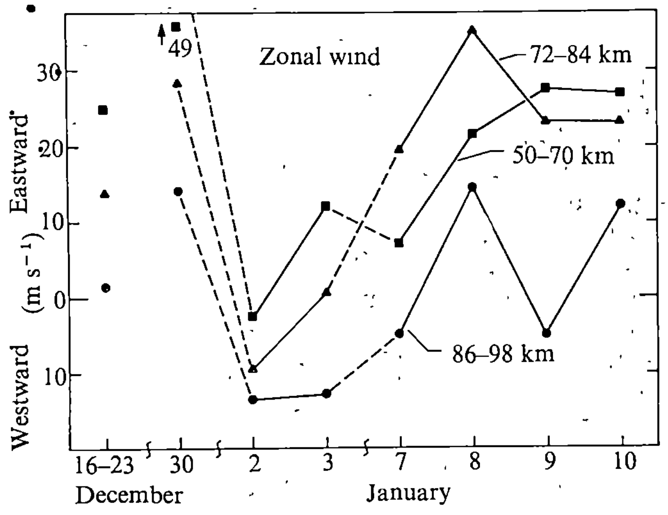

Fig. 3 Zonal component of daily median wind, around noon, in the altitude ranges 50–70, 72–84 and 86–98 km, at Ottawa: (Note the breaks in the sequence of dates.)

By January 1, the zonal wind below 95 km had reversed (and now blew to the west), and the flow above 95 km also altered significantly (Fig. 1). By January 5, recovery had commenced, though to reach speeds lower than before the warming. (A second warming was observed in early March, but is not discussed here.)

The extent to which wind changes at various altitude intervals are related, is demonstrated in Fig. 2. From the daily sequence of observations at Saskatoon, 21-d sequences have been selected, each starting a day after the previous sequence. Cross-correlation coefficients have been computed between the lowest altitude interval, 52–70 km, and successively higher intervals, for each 21-d sequence, with zero relative delay. (Coefficients of value 0.41 are significant at the 5% level, and those of value 0.53 at the 1% level.) The sequence of correlation coefficients shows that coupling existed between altitude intervals up to 85 km by about December 17, and to 100 km around the period of maximum stratospheric change, January 9–17.

Observations at Ottawa also show the effects of the warming. For present purposes, only those observations between December 30 and January 10 are considered. Figure 3 shows the mean zonal speeds, in three height ranges, 50–70, 72–84 and 86–98 km, derived from 40-min records around noon. This figure shows that reversal of the zonal flow, 50–98 km, occurred between December 30 and January 2, and that the initial flow was not resumed until January 8. These dates agree substantially with those for Saskatoon and Churchill.

Our observations, together with others at Saskatoon for the winters 1969–74, and the Alaskan observations[8], show that changes in mesospheric and lower thermospheric winds can be expected during stratospheric warmings, as can, presumably, temperature changes. Aeronomical changes are undoubtedly a consequence of these dynamical changes. In future studies, simultaneous observations of both dynamical and aeronomical conditions, on a spatial scale adequate to establish transport effects, will be required.

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Cavitation damage in dilute polymer solutions

HERE we report that the addition of small quantities of high molecular weight, water-soluble polymers to water (in our case polyacrylamide) increases the rate of vibratory cavitation erosion of copper. This result, together with that of Kudin[1], who obtained an enhanced rate of jet cutting after a similar addition, strengthens the analogy drawn between the two processes, and indicates that microjets may play a significant role in cavitation damage. These results may well be relevant for rain erosion, a process often compared with cavitation and jet cutting.

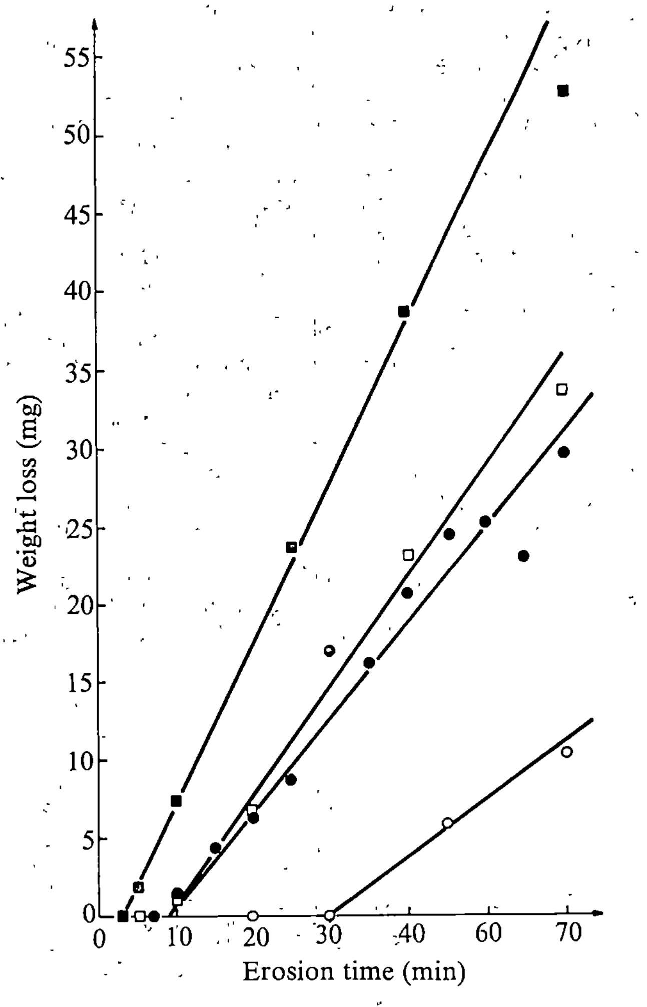

Fig. 1 Curves of vibratory cavitation erosion weight loss as a function of time for copper specimens in: ●, distilled water; □, equilibrated 100 w.p.p.m. polyacrylamide solution; ■, equilibrated 1,000 w.p.p.m. polyacrylamide solution; ○, 2:3 v/v glycerol–distilled water mixture.

Table 1 Experimental details

Solution	Conductivity (μS) (1 Siemen = 1mho)	*p*H	Viscosity (cpoise)	Surface tension (dyne cm^{-1})
Distilled water	3.0	5.5	0.89	72.5
Fresh 100 w.p.p.m. polymer solution	8.7	5.5	2.14	72.7
Equilibrated 100 w.p.p.m. polymer solution	9.7	—	1.31	—
Fresh 40 w.p.p.m. polymer solution*	—	—	1.29	—
Fresh 1,000 w.p.p.m. polymer solution	—	—	(26)	73.5
Equilibrated 1,000 w.p.p.m. polymer solution	4.5	5.1	3.85	71.0
Fresh 200 w.p.p.m. polymer solution*	—	—	4.05	—
Glycerol–distilled water mixture (2:3, v/v)	10.5	6.0	3.99	68

*These data are included for comparison purposes, to give an indication of the extent of polymer degradation, in terms of loss of viscosity.

Kudin's result[1] seemed to be of particular relevance to the mechanism of cavitation erosion of metals, since one model suggests that damage occurs as the result of the asymmetric collapse of cavities, leading to the formation of high velocity microjets which impinge on, and erode the metal surface (for example, refs 2 and 3). In view of their effects on jet cutting efficiency, it would therefore be anticipated that the presence of these polymers in a cavitating liquid would result in increased cavitation erosion. It has been shown in water tunnel experiments of Ting[4], however, that polyethylene oxide in tap water inhibits the inception of cavitation. Although this result does not give any indication of the effect on the rate of erosion once cavitation is produced, he[4] further suggests that, after the onset of cavitation, the asymmetric collapse of cavities and the formation of liquid microjets will also be retarded. It is postulated that this would be the result of increased resistance to flow, created by the viscoelasticity of the polymer solution[5]. If this latter suggestion is correct, it would be anticipated that the addition to cavitating liquids of soluble polymers, with appropriate viscoelastic properties, would result in decreased cavitation erosion.

It should be noted that an alternative model suggests that cavitation erosion is caused by the shock waves which result from the approximately symmetrical collapse of bubbles. It can be shown, however, that any increase in viscosity should have a damping effect on the rates of cavity growth and collapse. This would tend to reduce the magnitude of the shock wave, and to result in a decreased rate of cavitation erosion.

The cavitation experiments were undertaken on cold-drawn OFHC copper; the specimens used were disks, 19 mm in diameter and 6.4 mm thick, which were mechanically polished with 6 μm diamond paste. The tests were carried out in distilled water, and in solutions containing 100 w.p.p.m. and 1,000 w.p.p.m. of a water-soluble polyacrylamide (Percol 139, an anionic copolymer of acrylamide and 5% acrylic acid) with a mean molecular weight of about 10^7. Cavitation was produced by an ultrasonic probe (frequency 20 kHz) positioned immediately above the stationary copper specimen. In all tests, the titanium tip of the probe was 35 mm below the surface of the test solution, the amplitude of vibration was 60 μm, and the separation was 1.3 mm. The bulk temperature of the test solution was maintained at 298 ± 1 K, but no attempt was made to exclude air.

Preliminary experiments showed that the polymer in solution was partially degraded by extensive exposure to cavitation. A continuously refreshed liquid system, with a flow rate of 10^{-4} m^3 min^{-1} into a test vessel with a capacity of 2×10^{-3} m^3, was therefore used. By this means an 'equilibrium' solution, with a constant viscosity, was obtained. Before the start of each cavitation run, the initial solution in the test vessel was degraded to the equilibrium viscosity by means of cavitation in the absence of the copper specimen. The viscosities of the polymer solutions used, before and after equilibration, are reported in Table 1.

Figure 1 shows the cavitation erosion weight loss–time curves for copper in distilled water, and in equilibrated solutions containing 100 w.p.p.m. and 1,000 w.p.p.m. polyacrylamide. With the exception of relatively long exposure times, the effect of

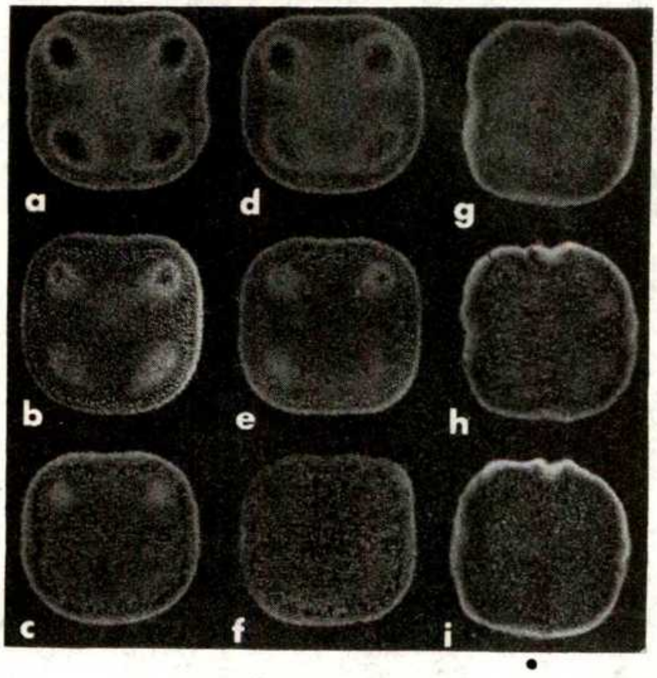

Fig. 2 Surface appearance of copper specimens after vibratory cavitation erosion in: *a*, distilled water for 10 min; *b*, distilled water for 20 min; *c*, distilled water for 60 min; *d*, equilibrated 100 w.p.p.m. polyacrylamide solution for 10 min; *e*, equilibrated 100 w.p.p.m. polyacrylamide solution for 20 min; *f*, equilibrated 100 w.p.p.m. polyacrylamide solution for 60 min; *g*, equilibrated 1,000 w.p.p.m. polyacrylamide solution for 10 min; *h*, equilibrated 1,000 w.p.p.m. polyacrylamide solution for 20 min; *i*, equilibrated 1,000 w.p.p.m. polyacrylamide solution for 60 min. Magnification ×2.2.

100 w.p.p.m. polymer additions is negligible. It is clear, however, that in the case of 1,000 w.p.p.m. polymer additions, the incubation time is reduced from ~ 8 to ~ 3 min, and the rate of weight loss is increased by a factor of ~ 1.4. These quantitative observations are confirmed qualitatively by the surface appearance of the specimens after erosion for equivalent times in the three liquids, shown in Fig. 2.

The data presented in Table 1 indicate the extent to which the polymer additions increase the viscosity of distilled water. It is widely accepted that, in the absence of other overriding factors, an increase in viscosity of the cavitating liquid results in less severe cavitation damage[6,7]. This effect has been confirmed for the present experimental conditions by undertaking cavitation tests on pure copper in a 2:3 (v/v) glycerol–distilled water mixture, which has a viscosity comparable with that of an equilibrated 1,000 w.p.p.m. polymer solution. The results of these experiments are also shown in Fig. 1. The increase in viscosity associated with the addition of glycerol to distilled water, increased the incubation time to ~ 30 min, and decreased the erosion rate by a factor of ~ 0.6 compared with that observed in distilled water. These data clearly confirm that the increased damage associated with polyacrylamide additions cannot be due to an increase in viscosity.

The data shown in Table 1 also indicate that the addition of soluble polyacrylamide does not significantly affect the surface tension of distilled water. The importance of these data lies in the fact that it has been shown that a marked decrease in surface tension, produced by the addition of a detergent, can also increase cavitation damage (V. A., D. Hulse, and R. P. M. P., unpublished).

Our result with additions of 1,000 w.p.p.m. polymer is in direct contrast to the theoretical predictions of Ting[4], but is fully consistent with the observed effect of such additions to jet cutting solutions.

The above results give no indication as to the mechanism by which the addition of polyacrylamide increases cavitation damage. It should be noted that, although the polyacrylamide does not significantly affect the surface tension of the solutions, it does increase markedly their viscosity. It has been shown experimentally, however, that an equivalent increase in viscosity, produced by the addition of glycerol, greatly decreases cavitation damage. Clearly, whatever the mechanism by which polyacrylamide additions increase cavitation damage, it is sufficient, in the case of the 1,000 w.p.p.m. solution, to outweigh the decrease in cavitation damage which would be expected on the grounds of viscosity alone. In the case of the 100 w.p.p.m. solution, it is possible that the two opposing effects balance each other.

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Anomalous thermal behaviour of induced phosphorescence in irradiated, doped polymeric glasses

WE report here that the intensity of induced phosphorescence in pre-irradiated, doped polymethylmethacrylate (PMMA) glasses changes in an anomalous way with temperature. At room temperature the irradiated part of the sample shows intense phosphorescence after ultraviolet excitation with an inspection lamp[1], whereas the part of the sample not previously irradiated shows no afterglow. When, however, the sample is cooled to the temperature of liquid nitrogen and the ultraviolet photoexcitation is repeated, the irradiated spot appears dark on the bright afterglow background of the rest of the sample (Fig. 1*a* and *b*). In Fig. 1*a* the central, ultraviolet irradiated, spot is seen as a bright

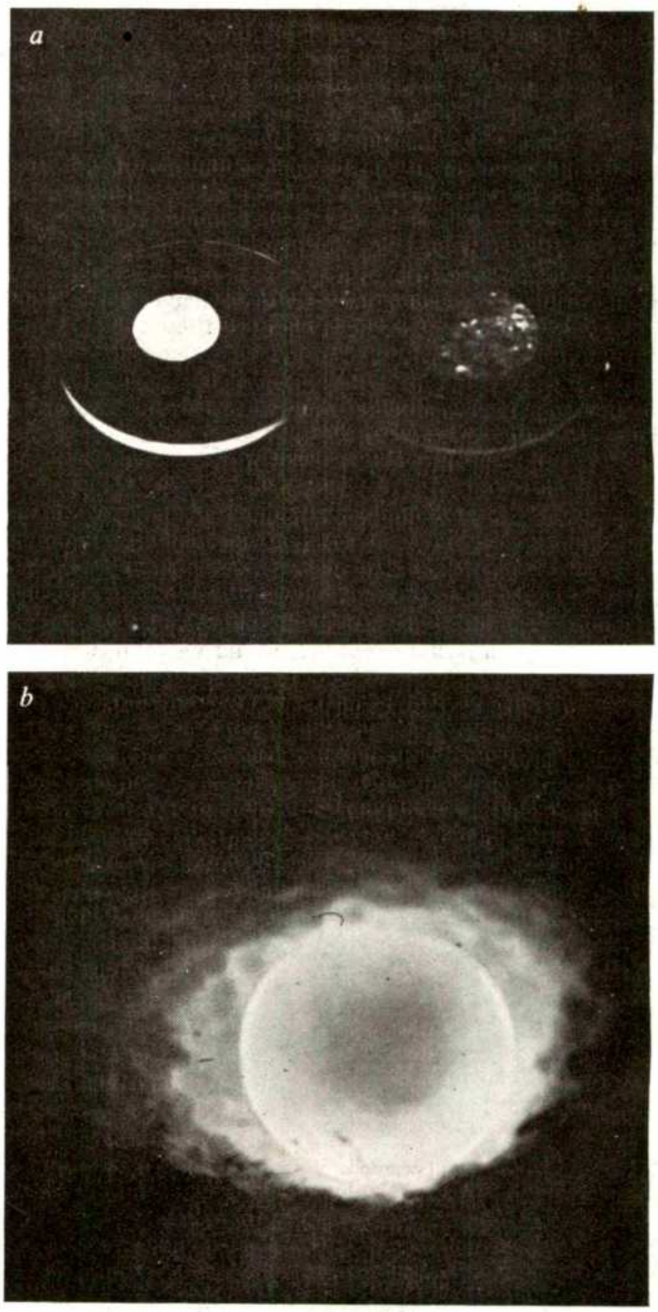

Fig. 1 *a*, Phosphorescence at room temperature after ultraviolet excitation has been switched off: left hand side, an X-ray irradiated sample; right hand side, an ultraviolet irradiated sample. X-ray and ultraviolet irradiated regions show clearly emission in the central areas of the samples; some of the emission is transmitted to the edges by a light guiding effect of the plane surfaces of the samples. The regions in the outer portion of the samples were not pre-irradiated and do not show room temperature luminescence. *b*, Phosphorescent glow in sample on the right of Fig. 1*a*, after it had been immersed in liquid nitrogen and the ultraviolet excitation had been switched off. The central area, which had been pre-irradiated with ultraviolet radiation, shows less phosphorescence than the area which had not been irradiated. As in Fig. 1*a* some light leakage is seen around the rim of the sample and in the liquid nitrogen.

circular region at room temperature after excitation with an ultraviolet inspection lamp; Fig. 1*b* shows the same irradiated spot, now relatively dark, after the sample has been cooled to 77 K and excited by the same inspection lamp.

Triphenylene is a convenient dopant because of its long triplet lifetime, 16 s, compared with dibenzothiophene which has a shorter lifetime of 1.5 s. Samples containing coronene as the dopant in PMMA after ultraviolet irradiation also show similar photographic results at room temperature and at low temperatures; visual inspections, using several dopants including dipbenzothiophene, dibenzothiophene sulfone and dibenzofuran have confirmed this. Quantitative studies have also confirmed that the induced phosphorescence shows an anomalous temperature–intensity relationship. The variation in the peak intensity of phosphorescence (measured at 462 nm) over a range of temperatures down to 188 K, for samples of non-irradiated polymethylmethacrylate doped with dibenzothiophene is shown in Fig. 2*a*. The curve is typical of those obtained for a number of different samples; because of variations in the thermocouple position slight shifts have been observed in the curves. The curves exhibit behaviour typical of normal phosphorescence in doped, organic glass matrices[2]. The accepted explanation of this effect is that as the temperature of the system is lowered the probability that non-radiative deactivation of the lowest triplet state (T_1) to the lowest state (S_1) will occur is reduced. This reduction is much greater than the reduction of energy transfer between higher singlet levels or between the first excited singlet state and the lowest triplet state. There is, therefore, still an appreciable energy transfer taking place between S_1 and T_1 so as to enable a certain number of triplet levels to be populated. These will then be emptied, increasingly, by radiative transitions, and thus phosphorescence is observed.

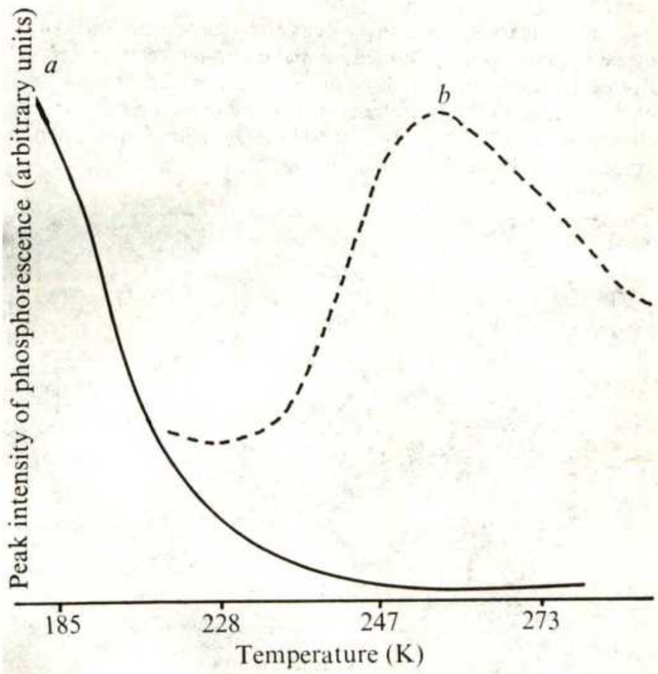

Fig. 2 *a*, Peak intensity of phosphorescence against temperature for a non-irradiated sample of doped PMMA; *b*, induced intensity of phosphorescence against temperature for an irradiated, doped sample of PMMA.

The behaviour of the induced phosphorescence peak of ultraviolet irradiated polymethlmethacrylate doped with dibenzothiophene is shown in Fig. 2*b*. Curves from a number of similar experiments show slight shifts in maxima; this is probably caused by variations in the position of the thermocouple with respect to the emitting surface. The main distinctions between Fig. 2*a* and *b* are, first, a non-irradiated sample does not show room temperature phosphorescence, whereas an irradiated sample shows an appreciable intensity of phosphorescence at room temperature; second, unlike the non-irradiated sample, the intensity of phosphorescence of the irradiated sample does not continue to rise as the temperature is reduced; it shows a maximum in the region of 250 K, after which the intensity falls as the temperature is further reduced. At about 228 K the intensity of phosphorescence of the irradiated sample falls to a value that is about the same as that of the non-irradiated sample. Further it does not show a marked increase as the temperature continues to be reduced to 185 K. This is contrary to the behaviour of non-irradiated samples.

A full explanation of this behaviour cannot be given at present; however, some of the features can be explained. Three temperature regions can be identified. Region I, from room temperature down to approximately 240 K, starts with significant phosphorescence at room temperature and increases to a maximum. We have postulated[1,3,4] that radicals or traps derived from PMMA, are involved in energy transfer from PMMA to the triplet state and that there is no exclusive requirement for population through the excited singlet state of the dopant. If it is assumed that all non-radiative processes diminish as the temperature is decreased, then curve *b* (region I) in Fig. 2 is consistent with the usual temperature behaviour for phosphorescence in photoinert matrices. The intensity in PMMA, moreover, is very substantially higher because of direct population of the triplet state bý energy transfer. In temperature region II, from approximately 240 K down to approximately 230 K, the induced phosphorescence decreases sharply, in contrast with the behaviour for a photoinert matrix. Assuming, as is reasonable, that the rates of the radiative processes are unaffected, but that the non-radiative decay processes are slightly decreased, it seems that energy transfer between the PMMA radicals and the triplet levels is involved.

The energy transfer which seems to diminish considerably in region II may be affected, possibly by the progressive freezing out of the diffusional mobility of the radicals or, more probably, by a reduction in the extent of the appropriate geometrical orientation of energy transfer partners. PMMA is known to have a γ transition at 268 K, attributed to hindered rotation of the ester group[5]. Temperature range III, below 230 K, shows a slight increase in phosphorescence. It can be assumed that energy transfer has been reduced to a minimum and the increase would then correspond to the further reduction of non-radiative processes from the triplet level. The intensity then lies below that for a photoinert matrix and much of the input energy is therefore degraded in the matrix (Fig. 2).

The enhancement of phosphorescence at room temperature is a striking and useful phenomenon though it does not persist below 230 K in this particular system.

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Measurements of erythemally effective ultraviolet radiation and total ozone content

MEASUREMENTS of erythemally effective ultraviolet radiation—wavelengths between 290 and 320 nm—are now being made at Aspendale (38°S, 145°E) and Brisbane (27°S, 153°E) as part of separate studies being undertaken by research groups in Australia and the USA. The following preliminary analysis of these measurements supplements the theoretically calculated relationships[1-4] between the total atmospheric ozone content and the intensity of ultraviolet radiation incident at the Earth's surface.

The data were obtained at Aspendale between May 1966 and February 1970, using an instrument described by Robertson[5], and between June and August 1974 with two instruments—one described by Urbach *et al.*[2] and one developed by CSIRO; the Brisbane data were obtained between May and July 1974, also using the instrument of Urbach *et al.* The three instrument types are similar in operation, and have almost identical spectral responses. Each unit prints the half-hourly totals of integrated erythemal effectiveness of the ultraviolet radiation. The present analysis was performed for cloudless days only, and these days were selected from continuous records of global and diffuse short-wave radiation at Aspendale, and from visual reports of cloud cover at Brisbane. For each set of data listed in Table 1, a graph showing the daily maximum half-hourly total of ultraviolet radiation (the solar noon ultraviolet irradiance) was plotted against time, and a line of best fit drawn by eye. The graph for Aspendale is shown in Fig. 1*a*. In addition, graphs were plotted of the mean annual variation of total ozone content, from eighteen years of data for both Aspendale and Brisbane. The Aspendale curve is shown in Fig. 2*b*. The departures of both total ozone content and the noon ultraviolet irradiance from their mean values, were then expressed as percentages, and plotted against each other as in the example of Fig. 2 for Aspendale 1966–70. A line of best fit was computed using a method of least squares, in which the ozone and ultraviolet measurements were assumed to have similar relative errors[6]. For each set of data, the slope of the line of best fit is a measure of the change in the ultraviolet irradiance, which results from a change in total ozone content. These measured dependences are given in Table 1.

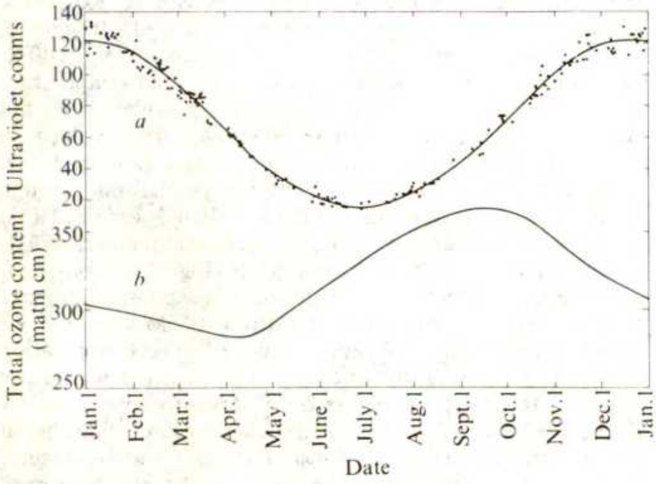

Fig. 1 *a*, Maximum half-hourly ultraviolet total for clear days at Aspendale, May 1966–February 1970. *b*, Mean annual variation of total ozone content at Aspendale.

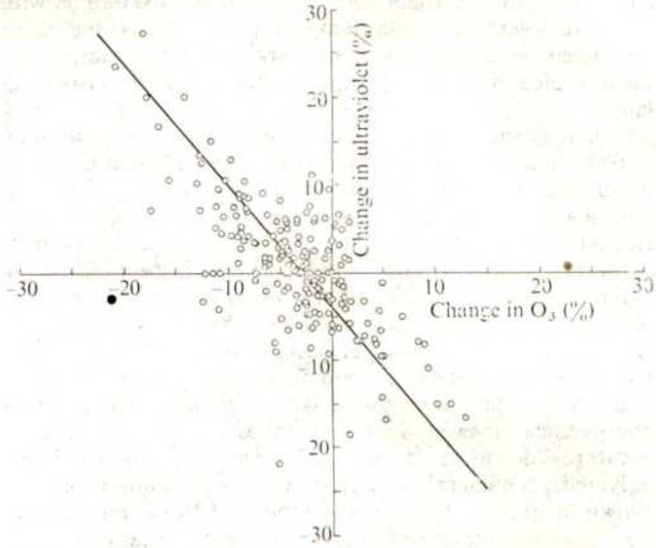

Fig. 2 Percentage change of ultraviolet intensity from the mean value as a function of percentage change of ozone content from the mean value. The graph is for the Aspendale (1966–70) data. ———, Line of best fit.

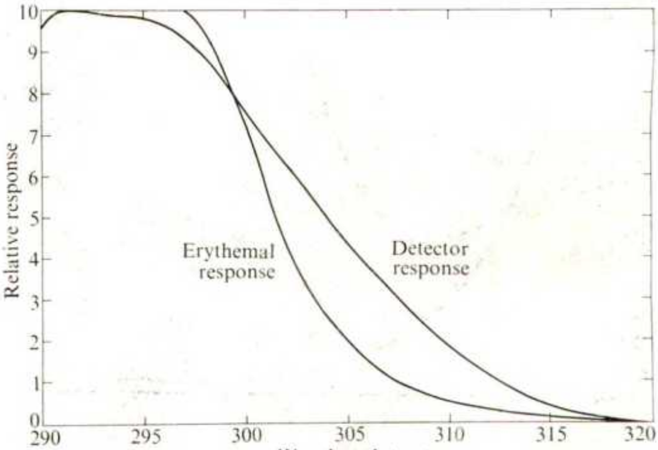

Fig. 3 The spectral response of the detectors. and the erythemal response of human skin.

In the table, the measured dependences are compared with those calculated using the equations and semi-empirical parameters of Green *et al.*[7]. These equations and parameters, which are based on the results of Bener (unpublished) give the ultraviolet irradiance at individual wavelengths in the range 280 to 340 nm as a function of solar zenith angle and total ozone content. The expected increase in 'measured' irradiance, for a total

Table 1 Results of clear day analysis

Location	Period	Instrument	No. of cloudless days	Ultraviolet dependence on total ozone content* Measured	Calculated Detector	Calculated Erythemal
Aspendale	May 66–Feb. 70	Robertson[5]	184	−1.40	−1.4	−1.7
Aspendale	June 74–Aug. 74	CSIRO	18	−1.32	−1.6	−1.8
Aspendale	June 74–Aug. 74	Urbach *et al.*[2]	22	−1.36	−1.6	−1.8
Brisbane	May 74–July 74	Urbach *et al.*[2]	31	−1.03	−1.4	−1.6

*The figures given are the ratios of percentage change in ultraviolet radiation intensity to percentage change in total ozone content.

ozone decrease of 10%, was then obtained by combining the calculated spectral irradiance with the spectral response of the detectors (Fig. 3). In addition, the dependences of erythemally effective ultraviolet irradiance on the total ozone content were calculated as above, but using the actual erythemal response curve given in Fig. 3. For the Aspendale 1966–70 data there is excellent agreement between the measured and calculated values. The other three periods of measurement give less satisfactory agreement, presumably because of the limited amount of data.

The Australian monitoring network now includes four stations; and further stations are planned. The importance of such long term monitoring programmes in this band of radiation is shown by the recent concern regarding the effects of possible anthropogenic modification of the stratospheric ozone layer (see, for example, ref. 8).

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Movement of the hyoid apparatus during chewing

The movements of the hyoid bone and the larynx during swallowing are only well established for man[1–3]. Their movements, if any, during mastication in man and in other mammals have been neglected. The hyoid is suspended from the cranium, above and behind, by the posterior suprahyoid muscles (stylohyoid and the posterior belly of digastric) and from the symphyseal region of the jaw in front by the anterior suprahyoid muscles (anterior belly of digastric, mylohyoid, geniohyoid and the more inferior fibres of genioglossus). The infrahyoid, or 'strap' muscles of the neck connect the hyoid with the sternum directly by the sternohyoid and indirectly through the thyroid cartilage of the larynx (thyrohyoid and sternothyroid) as well as with the scapula (omohyoid). This arrangement of the hyoid apparatus is found in all mammals, except that the digastric may not have a direct hyoid connection[4]: a situation occasionally found in man[2]. These three groups of muscles (anterior and posterior suprahyoids and infrahyoids) are recognised, on the basis of anatomical, cinematographic[5] and electromyographic[6] studies of human deglutition, as acting in combination first to elevate and then to depress the hyoid and larynx in swallowing. The posterior suprahyoids and the infrahyoids are also classically regarded[1,2] as acting to "stabilise"[1] or "fix"[2] the hyoid so that the anterior suprahyoids, and particularly the anterior belly of digastric[6,7], can pull the symphyseal region of the jaw towards the hyoid and open the mouth. Mandibular depression follows the power stroke[8] of every chewing cycle. The classical view of the hyoid apparatus is of a somewhat immobile bone and a group of muscles whose only active role is in swallowing. This concept may have led to the view that, since the omohyoid in man made such an uncertain contribution to hyoid movement or control of hyoid position, a "specific"[1]

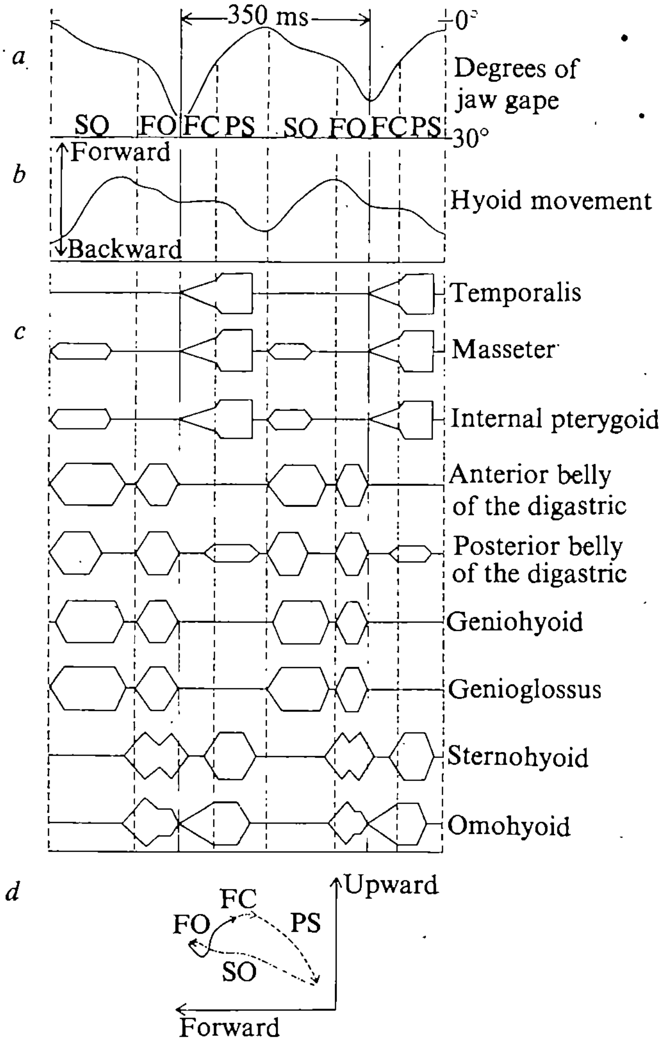

Fig. 1 Jaw and hyoid movement (*a*, *b*) in the opossum during part of a typical sequence of chewing on soft food. In *a*, the relative movement of upper and lower jaws has been plotted as degrees of gape (vertical axis) against time (horizontal axis). Each chewing cycle has four stages: FC, fast closing; PS, power stroke, (in this case consisting almost entirely of a slow closing movement); SO, slow opening; and FO, fast opening. In *b* the movements of the hyoid bone have been plotted on a plane parallel to the secondary palate; the range of antero-posterior movement is of the order of 20 mm. The associated EMG activity in adductor and representative hyoid muscles is shown in *c* as the envelope of the electrical signal. *c*, Composite diagram based on data from a large number of experiments. The path of movement of a point on the hyoid during chewing is shown in *d*. The diagram was obtained by plotting the position of the point in the 'vertical' and 'horizontal' planes (with reference to the hard palate, see text) at equal intervals of time during the cycle shown in *a*. Small skin incisions were made in anaesthetised animals so that bipolar wire EMG electrodes[9] could be inserted into the jaw elevators[10] and the major hyoid muscles (both bellies of digastric, geniohyoid, genioglossus, sternohyoid and omohyoid) under direct vision. On recovery, the animals were fed a minced proprietary canned dog food. Cinefluorographic recordings of jaw and hyoid movement were made in the lateral projection at 60 frames s^{-1} on 16-mm film[8]. EMG activity was recorded simultaneously on magnetic tape, together with a frame-by-frame synchronisation signal from the cine camera. The position of the jaws was measured on each frame of film as degrees of gape[11]. The position of the hyoid was measured separately in both the vertical and antero-posterior planes: for the former, the palatal line was used as a reference plane and for the latter, a perpendicular from that plane through the squamo-dentary joint. The tape recordings of the EMG activity were then converted into paper records and the positions of both the jaw and the hyoid were plotted on to these records: the data from each frame of film were registered against each synchronisation signal and a movement profile obtained.

and "speculative"[2] function should be attributed to this muscle; namely the tensing of the cervical fascia to assist prolonged inspiration during forced respiration. The latest edition of *Gray's Anatomy*[2] comments, "so far no useful data in regard to the hyoid musculature as a whole have been contributed by electromyographers" (page 508).

The American opossum (*Didelphis virginiana*), which is regarded as exemplifying both early mammalian cranio–facial organisation in general and the mammalian hyoid apparatus in particular, has been studied by synchronised cinefluorography and electromyography (see legend to Fig. 1).

Jaw movement in the chewing cycle of the opossum is shown in Fig. 1*a*. The associated movements of the hyoid in the antero-posterior plane are also shown (*b*). Movement of the hyoid in both the horizontal and vertical planes is shown in Fig. 1*d*. The chewing cycle begins with the mouth wide open (maximum gape). At this point the hyoid is about a third of the way along its antero-posterior movement path. As the jaw is elevated rapidly, the hyoid moves very slightly upwards but may not significantly change its antero-posterior position. Both the fast closing movement of the jaw and the elevation of the hyoid cease at tooth–food–tooth contact. As the jaw is moved upwards against the resistance of the food in the 'slow close' of the power stroke, high amplitude EMG activity (Fig. 1*c*) is seen in the elevator muscles[12,13]. At the same time, high amplitude activity in the sternohyoid and omohyoid muscles is associated with downwards and backwards movement of the hyoid. No electrical activity was recorded in the anterior suprahyoid muscles during the power stroke. On completion of the power stroke, the jaw has reached its maximum elevation and the hyoid its most inferior and posterior position. The opening stroke of the chewing cycle occurs in two stages; slow opening and then fast opening. In the first, the jaw is moved slowly downwards as the hyoid moves both forwards and upwards. These convergent movements of the jaw and hyoid are associated with activity in the anterior belly of the digastric, the geniohyoid and the genioglossus, all of which are acting to depress the lower jaw and elevate the hyoid simultaneously. There is a small burst of EMG activity in the posterior belly of digastric coincident with the initial early elevation of the hyoid. The sternohyoid and omohyoid are electrically silent during slow jaw opening and do not, therefore, prevent the hyoid from being drawn forwards. Low level activity in the jaw elevators, particularly the masseter and internal pterygoid was recorded during slow opening[12], when the tongue is collecting and repositioning food within the mouth. As the hyoid is moved forwards and upwards, the floor of the mouth and the base of the tongue are also raised by the contracting anterior suprahyoid muscles. The activity in these muscles tends to depress the jaw, which is partially counteracted by the activity in the elevators. Fast opening of the jaw occurs after the hyoid has reached its most anterior and elevated position at the end of slow opening. As the jaw is depressed, the anterior part of the tongue, protruded in slow opening, is retracted sharply. The hyoid is moved slightly upwards and backwards to its midway position. Rapid opening of the jaw is associated with high amplitude EMG activity in all the muscles of the hyoid apparatus.

Contrary to classical anatomical teaching, at no time during the chewing cycle is the hyoid 'fixed'. It shows least movement during the fast close stage of the cycle and most movement during opening. This is not what would be expected were the mandible depressed towards a 'fixed hyoid'. During slow closure in the power stroke, the hyoid moves downwards and backwards; in slow opening it is moved upwards and forwards. There are two important aspects of the pattern of muscle activity responsible for the hyoid cycle. First, the EMG activity in the anterior and posterior bellies of digastric is asynchronous, both in its time of onset and in its peak amplitude; this finding contrasts with the statement "that (in man) the two bellies must contract with equal force"[1]. Both bellies are, however, involved in fast opening when all the muscles of the hyoid apparatus are electrically active and are combining both to depress and retract the hyoid and to depress the lower jaw. Second, during slow opening, the infrahyoid muscles are not actively contracting and do not, therefore, 'stabilise' the hyoid.

In the light of these results, the classical view of the role of the hyoid apparatus must be revised. The hyoid has a cyclical movement completely integrated with that of the jaw and related to the role of the tongue in positioning the food. Further, the hyoid is part of a dynamic and not a basically static system. In man, the acquisition of the upright posture has resulted in some minor shifts in the angulation of the hyoid muscles compared with *Didelphis* but not in any significant change in their anatomy. The initiation and regulation of hyoid movement may, therefore, be seen as the normal function of the omohyoid. This does not, however, preclude the possibility that this muscle, whose anatomy in man has seemed somewhat inexplicable, occasionally assists in tensing the cervical fascia in the specialised respiratory state of prolonged inspiration[1,2].

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Fate of teratocarcinoma cells injected into early mouse embryos

ANALYSIS of early mammalian development is complicated by technical difficulties. The initial processes of cellular determination and differentiation in the mouse embryo take place in small populations of cells[1,2], and major embryogenic events occur after uterine implantation when the embryo is largely inaccessible. Recent work, however, suggests that murine teratocarcinomas may provide a convenient model for studying mammalian development[3–6]. These are transplantable tumours of germ cell or embryonic cell origin[3–6], typically consisting of a variety of differentiated tissues and undifferentiated stem cells. The stem cells, called embryonal carcinoma, resemble cells of

Table 1 Details of embryonal carcinoma cell lines

Cell line	Tumour of origin	Chromosomes of tumour		Mouse strain	GPI type	Pigmentation		Approximate number of generations in culture
		Sex	Number			Albino	Agouti	
C86†	88‡ from 8-d embryo	XX	41	C3H	$1^b/1^b$	$+/+$	A/A	4–18
C17†	17‡ from 7-d embryo	XX	40	C3H	$1^b/1^b$	$+/+$	A/A	10–12
SIKR-OSB§	OTT5568 from 3-d embryo	XO*	40*	129	$1^a/1^a$	$+/+$	A^w/A^w	45

*These data refer to the chromosomes of the cultured cells rather than to the tumour of origin.
†M.W. McB. and C.F.G., unpublished.
‡S. A. Iles and E. P. Evans, unpublished.
§Ref. 20.

early embryos in morphological, biochemical and cell surface properties, and in developmental potential[3–6]. They can be propagated in tissue culture to provide sufficient material for biochemical analysis. After inoculation into histocompatible adult hosts they form differentiated teratocarcinomas. They also differentiate *in vitro*[7,8] where the first stages of their differentiation seem to parallel normal embryonic development. We show here that embryonal carcinoma cells can participate in normal embryogenesis, thus providing further evidence for the validity of the use of these cultures as a model of normal embryonic development.

When mouse blastocysts are injected with genetically distinct embryonic cells[9], they develop normally and tissues of the resulting offspring are often chimaeric, consisting of cells of both donor and host origin. The developmental potential of injected cells can be ascertained from the distribution and genetic activity of their progeny in such chimaeras[10,11]. Brinster[12] injected cells from the ascitic form of an *in vivo* teratocarcinoma into blastocysts of a different genotype and obtained one overtly chimaeric mouse. The purpose of our study was to confirm and extend this work by injecting cells from *in vitro* cultures of embryonal carcinoma into blastocysts. We obtained normal chimaeric mice following injection of two different embryonal carcinoma cell lines. A third line colonised the embryo but the resultant chimaeric mice developed teratocarcinomas postnatally.

Table 2 Rate of development of blastocysts injected with embryonal carcinoma cells after transfer to females that subsequently became pregnant

Cell line injected	Stage of pseudopregnant foster mother	Foetuses recovered / Embryos transferred		Number chimaeric
		9.5 d	Term	
C86	2.5 d	13/18(2)*	—	3(+2?)
SIKR–OSB	2.5 d	—	4/9(1)	1
C17	0.5 d	—	9/13(1)	1
	2.5 d	—	34/58(6)	3
C86	0.5	—	5/12(2)	0
	2.5 d	—	69/93(13)	6

* Figures in parentheses refer to the number of foster mothers to which the embryos were transferred.

The genetic markers used to distinguish embryonal carcinoma cell progeny from the cells of the host embryo were isozymal differences at the glucose phosphate isomerase (GPI) locus (*Gpi-1*), albino or pigmentation, and white-bellied agouti (A^w) or extreme non-agouti (a^e). Details of the origin and characteristics of the cell lines are presented in Table 1. Cultured embryonal carcinoma cells derived from C3H teratocarcinomas were injected into blastocysts obtained by matings of $Gpi\text{-}1^a/Gpi\text{-}1^a$ albino CFLP random-bred mice (Anglia Laboratory Animals Ltd). The 129/Sv–*Sl*–^{i}*CP* derived cell line was injected into $Gpi\text{-}1^b/Gpi\text{-}1^b$ blastocysts obtained from matings of a^e/a^e mice of the AG/Cam-derived balanced lethal yellow (A^y/a^e) inbred line[13].

Clumps of 20 to 40 embryonal carcinoma cells were removed mechanically or with trypsin from the culture vessel, and injected into the blastocoelic cavities of 3.5–4-d blastocysts with the aid of a Leitz micromanipulator assembly[14]. After 1–3 h incubation at 37 °C, blastocysts were transferred either to the uterus of day 3 or to the oviduct of day 0 pseudopregnant females. The foster mothers were either killed on day 10 of gestation or allowed to carry their young to term. Chimaerism in postimplantation embryos and internal tissues of offspring was investigated by electrophoretic analysis of homogenates for GPI in starch gels[15]. External chimaerism in offspring was determined by inspection of the coat and eyes for the presence of pigmented melanocytes or agouti hairs. Material for histological examination was fixed in neutral formal saline, embedded in wax and sections were stained with haemalum and eosin or Masson's trichrome stain.

In a preliminary series of experiments involving C86 cells, the foster mothers were killed on day 10 of gestation and all conceptuses were separated into a trophoblastic and embryonic fraction. Thirteen normal living embryos were recovered, and no resorption sites were present. GPI analysis indicated the presence of a significant minor component of C86 derived isozyme in three conceptuses: in the embryonic portion of one,

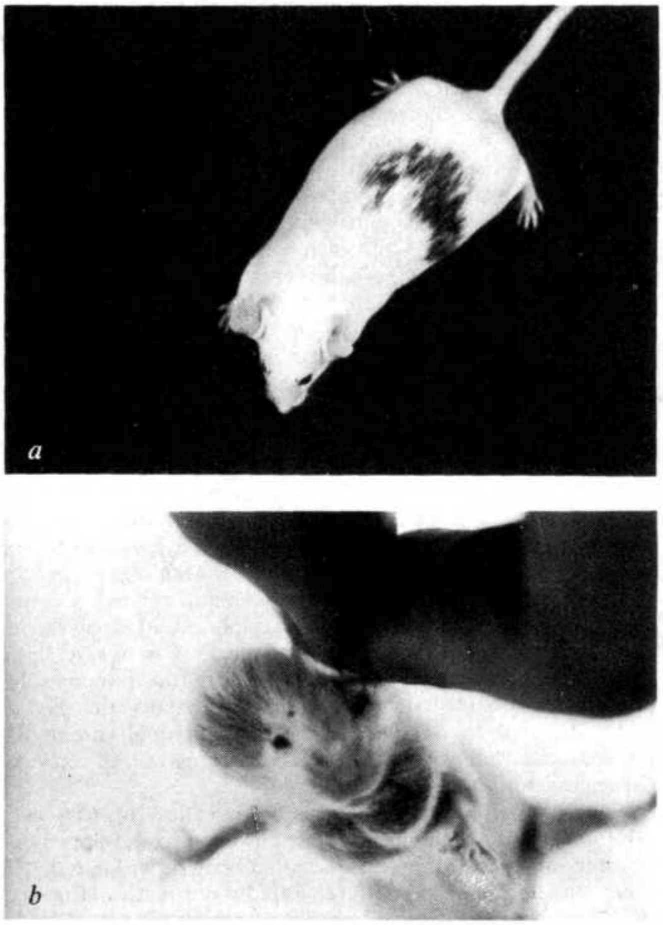

Fig. 1 Chimaeric animals. *a*, Animal T89 at 6 weeks of age showing pigmented area of coat. *b*, Animal T38 at 2 weeks of age showing three subcutaneous tumours in the neck and throat regions.

Table 3 Chimaeric animals

Animal code	Sex	Injected cell line	Animal fate	Age at death (d)	Pigment		GPI														
					Coat	Eyes	Blood	Brain	Gonads	Gut	Heart	Kidney	Liver	Lung	Skeletal muscle	Spinal cord	Spleen	Thymus	Tumour	Tumour location
T120	f	SIKR–OSB	k	42	C	?	C	H	C	—	C	C	H	C	C	—	H	C		None
T84	f	C17	l		H	C	H	—	—	—	—	—	—	—	—	—	—	—		None
T85	f	C17	l		C	C	H	—	—	—	—	—	—	—	—	—	—	—		None
T89	f	C17	l		C	C	H	—	—	—	—	—	—	—	—	—	—	—		None
T6	f	C17	d	7	C	C	C	H	—	C	C	H	H	C	C	H	H	—	C C	s.c. under ear s.c. base of tail
T18	f	C86	k	3	H	H	H	H	—	—	H	H	H	H	C	H	—	—	C	s.c. genital region
T33	f	C86	k	9	H	H	H	H	—	H	H	H	H	H	C	H	H	H	C	cerebellum
T38	f	C86	k	14	H	H	C	H	—	C	C	C	H	C	C	—	H	C	C C	s.c. under ear s.c. throat
T43	m	C86	d	8	H	H	—	H	C	—	H	H	C	—	C	—	C	—	C C	s.c. throat s.c. neck
T65	f	C86	d	26	H	H	H	H	—	—	H	H	H	H	H	—	H	—	C C	s.c. thorax s.c. below eye
T72	m	C86	d	5	H	H	—	H	H	H	H	H	H	C	C	—	—	—	C C	abdominal cavity s.c. under ear

k, Killed; d, died; l. live; H, host; C, chimearic; —, not analysed; ?, not analysable; s.c., subcutaneous.

the trophoblast of a second, and in both portions of a third. Two other conceptuses showed traces of C86-type isozyme. These results indicated that the injected embryonal carcinoma cells had proliferated in some embryos without obviously interfering with their morphogenesis. In subsequent experiments the embryos were allowed to develop to term.

The rate of development of operated blastocysts (Table 2) compares favourably with that found in previous experiments[14] and argues against embryonal carcinoma cells adversely affecting prenatal development in spite of the relatively large number of cells injected into each blastocyst. Of 121 mice born, 11 contained progeny of the embryonal carcinoma cells (Table 3) and 4 of these were normal healthy animals.

Mouse T120, obtained after injection of SIKR–OSB cells, had small patches of agouti hair in the coat, indicating the presence of embryonal carcinoma derived dermal elements interacting in the normal manner in the hair follicles[16]. This animal was healthy when killed at 6 weeks of age. Analysis indicated extensive chimaerism of internal tissues.

Mice T6, T84, T85, and T89 were obtained after injection of C17 cells. T6, T85 and T89 exhibited pigmentation in both the coat and eyes whereas T84 showed only eye pigmentation. Three remained apparently healthy at 9 weeks of age and the areas of pigmentation have proved stable in relative size and distribution (Fig. 1*a*). Apart from blood, GPI analysis for internal chimaerism of these three animals has not yet been undertaken as they are being test bred. The fourth mouse, T6, developed two subcutaneous tumours postnatally and died at 7 d. GPI analysis demonstrated that embryonal carcinoma-derived cells were present in several macroscopically normal tissues in addition to both tumours.

None of the mice obtained after injection of C86 cells exhibited coat or eye pigmentation; however, six developed one or more tumours which were evident at birth or within 6 d. All except one tumour (in T65) grew rapidly postnatally (Fig. 1*b*), so that all six mice died or were killed during the first 3 weeks of life. All tumours were subcutaneous except one that protruded through the skull from the cerebellar region of the brain and one attached to the liver. The tumours were poorly differentiated teratocarcinomas, consisting chiefly of embryonal carcinoma; GPI analysis indicated the presence of both embryonal carcinoma-derived and host cells. In addition, five of the six animals showed chimaerism of other macroscopically normal tissues, probably indicating the presence of normal, appropriately differentiated cells derived from the embryonal carcinoma. Because of the tumours, however, we cannot eliminate the possibility of numerous small metastases.

Normal embryonic cells can be converted into teratocarcinomas by transplanting embryos of various developmental stages into ectopic sites in syngeneic adult hosts[17–19]. Present experiments suggest that such teratocarcinoma-derived embryonal carcinoma cells taken from culture *in vitro* can participate in normal morphogenesis and differentiation when restored to the environment of the early embryo. We have obtained unequivocal normal chimaeras following the injection of pluripotential cells of two different lines. Embryonal carcinoma progeny in these chimaeras were able to differentiate into apparently normal functional melanocytes of both the coat and iris (T84, T85, T89) and normal dermal elements of hair follicles (T120). In future experiments we intend to use genetic polymorphisms of differentiated functions to investigate whether the embryonal carcinoma progeny in the other chimaeric tissues are functioning like normal differentiated cells of those tissues. Also we are, at present, attempting to increase the level of chimaerism by injecting dissociated cells directly into the inner cell mass of blastocysts to enable more effective interaction between embryonal carcinoma and embryo cells.

All living mice from the present experiments are being test bred to determine whether the embryonal carcinoma cell progeny can colonise the germ line and produce functional gametes. This would raise the possibility of producing mice from a tissue culture cell line selected to carry various mutations and of establishing lines of mice which are models for genetically determined human diseases, or which carry mutant alleles of relevance to studies on development.

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Note added in proof: Chimaeric mice have been obtained following injection of teratocarcinoma cells from *in vivo* tumours. One chimaera sired 61 offspring, all of which carried genetic markers of the teratocarcinoma cells[21].

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Collagen characterisation and cell transformation in human atherosclerosis

COLLAGEN comprises 40–60% of the protein[1,2] and 30% of the weight[3] of human fibrous atherosclerotic plaques. Its deposition in the arterial intima is largely responsible for the occlusive and irreversible nature of coronary and cerebral arterial disease. Four genetically distinct types of collagen have been identified in mammalian tissues and at least five structural genes are involved in their synthesis[4]. The transcription of these various genes seems to be tissue specific. Type I collagen is found in tendon, bone and adult skin, type II is found in cartilage and intervertebral disk, and type IV is restricted to basement membrane. Type III collagen was observed first in newborn human skin[5] and has been isolated and characterised from human aorta and leiomyoma[6]. Initial reports identified both types I and III in human aorta[4,7]. We have now found that fibrous atherosclerotic plaques contain proportions of these two collagens which are different from those occurring in normal arterial wall.

Eight aortas each containing large areas of fibrous atherosclerosis were obtained *post mortem* from middle-aged men (mean age 52 yr), washed free of blood and the adventitia stripped and discarded. Raised white intimal plaques were removed and pooled. Residual segments of aortic media were also pooled. The resulting two fractions were treated separately, but identically, in all subsequent procedures.

Minced, defatted tissues were first decalcified by exhaustive stirring with disodium EDTA. Collagen was then extracted from the washed decalcified samples by stirring for 24 h at 4 °C with 50 volumes of 0.5 M acetic acid containing 200 mg pepsin (Worthington PM, 2,800 U mg^{-1}) per g of lyophilised tissue[8]. Collagen was precipitated from the clarified digest by addition of solid NaCl to a concentraction of 0.9 M, redissolved in 0.5 M acetic acid and dialysed exhaustively against 1.0 M NaCl, 0.05 M Tris, *p*H 7.5. Initial separation of types I and III in each sample was achieved by raising the NaCl concentration in steps of 0.2 M up to 3.0 M. A substantial precipitate of type III collagen was obtained from the aortic medial extract at 1.6 M NaCl, but with atherosclerotic intima the initial precipitate was slight and came down at 1.8 M. At 2.6 M NaCl large amounts of type I collagen were obtained from atherosclerotic intima and smaller amounts from the media.

Samples (20 mg) of each freeze-dried, salt-fractionated precipitate were chromatographed on carboxymethylcellulose (see Fig. 1 for conditions) after denaturation at 45 °C for 40 min. Elution positions of rat skin collagen α1(I), α2 and β12 chains on carboxymethylcellulose are shown in Fig. 1*a*. The 1.6 M NaCl precipitate from aortic media yielded only a trace amount of α1(I). Instead, a broad peak eluted in an intermediate position between β12 and α2 (Fig. 1*a*).

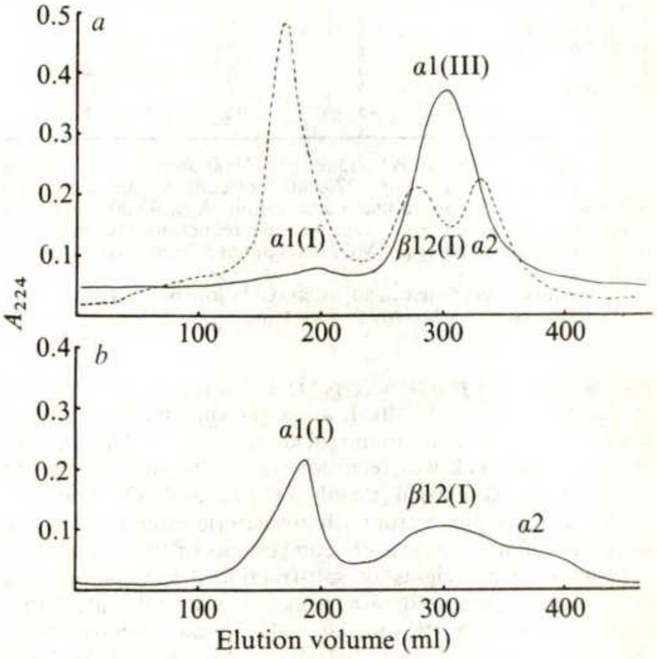


Fig. 1 Carboxymethylcellulose chromatography of aortic collagens on a 16×100 mm column at 40 °C in 0.02 M sodium acetate buffer containing 1 M urea, *p*H 4.8, using a linear gradient of 0.02 M to 0.12 M NaCl over a total volume of 500 ml. *a*, 1.6-M salt fraction from pepsin-treated human aortic media (——) and lathyritic rat skin collagen (– – –). *b*, 2.6-M salt fraction from pepsin-treated human atherosclerotic intima.

The latter component, rechromatographed on an Agarose column previously calibrated with collagen chains and peptides, yielded two major peaks eluting at positions corresponding to V_0 and 270,000 daltons (Fig. 2). Amino acid analysis (given in Table 1) of both major Agarose peaks was identical and seemed very similar to analyses reported for human α1(III)[9]. When the 270,000 molecular weight Agarose peak was reduced and alkylated and rechromatographed, a new component was produced with a molecular weight of approximately 95,000 and an amino acid composition corresponding to that of the original peak (Table 1). This suggested that much of the 270,000-dalton material was in the form $[\alpha1(\text{III})]_3$, held together with interchain disulphide bonds between adjacent cysteine molecules.

Although little collagen was precipitated from medial extracts by 2.6 M NaCl, large amounts were obtained from atherosclerosis intima. The carboxymethylcellulose chrom-

Table 1 Amino acid composition of collagen molecules and chains isolated from human aorta

Amino acid	Media $[\alpha 1(\mathrm{III})]_3$ Ag_1	Media $\alpha 1(\mathrm{III})$ Ag_2	Atherosclerotic intima $\alpha 1(\mathrm{I})$ CMC
3-Hydroxyproline	2	ND	ND
4-Hydroxyproline	127	126	104
Aspartic acid	48	49	41
Threonine	15	15	16
Serine	39	40	34
Glutamic acid	72	73	77
Proline	97	96	118
Glycine	350	367	342
Alanine	99	86	117
Cysteine	2*	1.6†	—
Valine	13	11	17
Methionine	8	7	6
Isoleucine	14	13	7
Leucine	22	22	19
Tyrosine	2	2	1
Phenylalanine	8	9	12
Hydroxylysine	7	7	8
Lysine	28	28	27
Histidine	6	5	3
Arginine	45	46	50

Results are expressed as residues per 1,000 amino acid residues. ND, not determined. Ag_1, 270,000 molecular weight peak from Agarose. V_0 peak had similar composition. Ag_2, 95,000 molecular weight peak obtained from Agarose after reduction, alkylation and rechromatography of Ag_1. CMC, α1(I) derived from carboxymethyl-cellulose.

*Determined as cysteic acid after oxidation by performic acid.

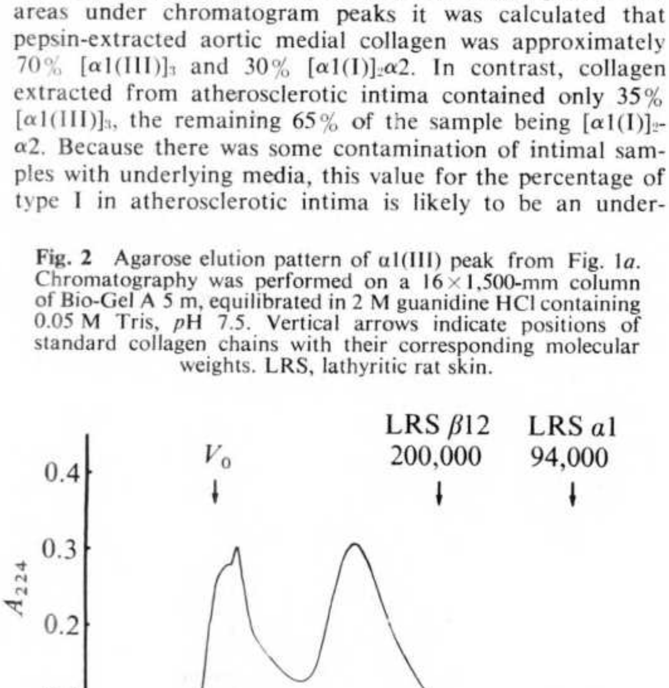
†Determined as carboxymethyl cysteine.

atogram of this fraction (type I) is shown in Fig. 1*b*. The initial peak was identified as α1(I) on the basis of its elution position and amino acid analysis (Table 1). The second broad peak was rechromatographed on Agarose and found to be composed mainly of β12 and α2. Thus the 2.6 M salt precipitate from atherosclerotic intima was found to contain almost exclusively components of type I collagen.

From known weights of salt-fractioned precipitates and areas under chromatogram peaks it was calculated that pepsin-extracted aortic medial collagen was approximately 70% $[\alpha 1(\mathrm{III})]_3$ and 30% $[\alpha 1(\mathrm{I})]_2\alpha 2$. In contrast, collagen extracted from atherosclerotic intima contained only 35% $[\alpha 1(\mathrm{III})]_3$, the remaining 65% of the sample being $[\alpha 1(\mathrm{I})]_2\alpha 2$. Because there was some contamination of intimal samples with underlying media, this value for the percentage of type I in atherosclerotic intima is likely to be an underestimate. The figures also assume that pepsin-solubilised collagen is representative of the whole.

The cells which synthesise these collagens are also different in the two sites. Those of the aortic media are well differentiated smooth muscle cells (SMC) similar to those shown in Fig. 3*a*. Our finding that the collagen of the

Fig. 2 Agarose elution pattern of α1(III) peak from Fig. 1*a*. Chromatography was performed on a 16×1,500-mm column of Bio-Gel A 5 m, equilibrated in 2 M guanidine HCl containing 0.05 M Tris, *p*H 7.5. Vertical arrows indicate positions of standard collagen chains with their corresponding molecular weights. LRS, lathyritic rat skin.



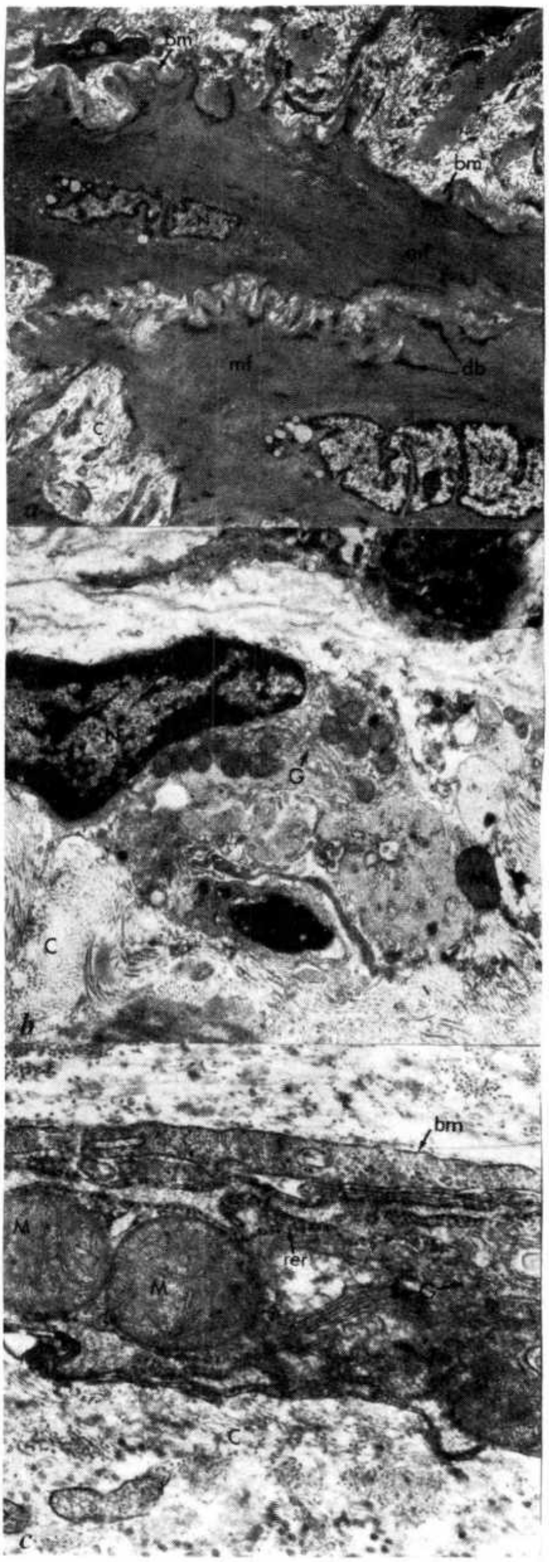


Fig. 3 Electron micrographs of cells from adult human aortae. Stained with uranyl acetate and lead citrate. *a*, Smooth muscle cells from media, × 3,330. *b*, Fibroblasts from atherosclerotic plaque, × 7,030. *c*, Fibroblast from atherosclerotic plaque, × 33,300. N, Nucleus; C, extracellular collagen; M, mitochondria; G, Golgi complex; mf, myofilaments; db, cytoplasmic dense bodies; bm, basement membrane; rer, rough endoplasmic reticulum.

aortic media is mainly type III, together with previous evidence of type III in leiomyomata[6], suggests that a mixed collagen in which $\alpha 1(\mathrm{III})$ is predominant is a characteristic product of SMC.

It is widely believed that SMC play an important role in atherogenesis[10,11]. There is considerable experimental evidence that many of the cells of the atherosclerotic plaque are derived from medial SMC which have migrated to the intima and proliferated. These cells often lose some of their distinguishing ultrastructural features and have in consequence been named myointimal cells[12] or 'modified' SMC[13]. If type III collagen could be used as a genetic marker of smooth muscle it would provide a functional criterion on which to base the identity of such cells.

In view of the supposed derivation of plaque cells from SMC the finding of large amounts of type I collagen in the intimal plaques was surprising. The pattern of synthesis is more similar to that of adult skin fibroblasts than it is to smooth muscle. Accordingly, we examined the appearance of the thickened aortic intima in the electron microscope. None of the plaque cells resembled SMC. Myofibrils, basement membrane and cytoplasmic dense bodies were absent. Instead, the cells were smaller and their cytoplasm was packed with ribosomes and organelles (see Fig. 3*b* and *c*) and they had the typical appearance of fibroblasts undergoing active protein synthesis[15].

If cells of human fibrous plaques are derived from smooth muscle, it is evident that they have undergone fundamental changes in both morphology and collagen synthesis. Since the new pattern of collagen synthesis would involve change in the rates of translation of genes coding for $\alpha 1(\mathrm{III})$, $\alpha 1(\mathrm{I})$ and $\alpha 2$, we suggest that a basic cell transformation takes place in human atherogenesis. An alternative explanation is that cells of the plaque are derived not from smooth muscle but from fibroblasts resting in normal intima. In that case transformation is unnecessary and it would be best to classify the cells as true fibroblasts. Further study is required to determine which of these interpretations correctly applies to human atherosclerosis.

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Tumour-specific antigen related to rat histocompatibility antigens

ATTENTION has been drawn to the similarities between tumour-associated antigens (TAA) and normal histocompatibility antigens, such as their high degree of polymorphism[1,2], their cell surface behaviour[3], and composition in terms of molecular weight and amino acid composition[4]. These similarities are reflected in methods developed for the isolation of tumour and histocompatibility antigens[5–7]. Furthermore an inverse relationship has been established between the expression of TAA and H-2 antigens on 3-methylcholanthrene-induced murine sarcomas[8,9], whereas the appearance of TAA on aminoazo dye-induced rat hepatomas was associated with deletion of cell surface-expressed normal liver-specific antigens[10].

One interpretation of these findings is that the individually characteristic TAAs on chemically-induced tumours may be modified histocompatibility antigens[11]. This hypothesis has been examined using a tumour-specific antigen preparation isolated from the serum of Wistar rats bearing transplants of a 4-dimethylaminoazobenzene-induced hepatoma (D23). This antigen, purified from immune complexes in tumour-bearer serum by gel filtration in acidic media followed by affinity chromatography on Sepharose 4B-linked syngeneic rat immune IgG, was homogeneous on isoelectric focusing gels in the presence or absence of neutral detergents. It also reacted specifically with antibody in syngeneic hepatoma D23-imune rat serum as shown by immunodiffusion in isoelectric focused gels and its capacity to neutralise membrane immunofluorescence staining of the serum with hepatoma D23 cells (J.G.B. and R.W.B., unpublished).

The highly purified hepatoma D23 antigen was injected intramuscularly in Freund's complete adjuvant into the hind limb of male New Zealand white rabbits, and after three injections at weekly intervals, the rabbit serum was tested by the indirect membrane immunofluorescence test for the presence of antibody directed against the tumour-specific antigen on viable hepatoma D23 cells (Table 1). The fluorescence index was calculated (FI = (% cells unstained with normal rabbit serum − % unstained with test serum)/% unstained with normal rabbit serum). Fluorescence indices of 0.30 or greater were taken to represent significant staining of hepatoma cells by antibody.

Table 1 Fluorescence indices of rabbit anti-D23 purified serum antigen tested against cells from three Wistar tumours

	Fluorescence index against target cells from:		
Serum	Hepatoma D23	Hepatoma D30	Hepatoma D33
Rabbit anti-D23 purified antigen	1.00, 0.97	1.00, 0.95	0.99, 1.00
Rabbit anti-D23 purified antigen absorbed *in vivo**	0.87, 0.79	0.01, 0.10	0.09, 0.07
Rabbit anti-D23 purified antigen absorbed by normal Wistar rat liver membranes†	0.75, 0.83	0.05, 0.03	0.13, 0.12

*Rabbit serum (2 ml) injected intraperitoneally into normal Wistar rats which were bled by cardiac puncture 4 h later.

†Serum (1 ml) absorbed with 50 mg normal Wistar liver membrane protein for 18 h at +4 °C.

The serum from rabbits immunised against the hepatoma D23 purified serum antigen reacted not only against hepatoma D23 cells (FI 1.00–0.97) but also against the two immunologically distinct hepatomas D30 and D33 (FI 0.95–1.00), indicating that this serum recognised antigenic similarities between these three tumours. After absorption *in vivo* or by isolated normal rat liver membranes, the reactivity of the rat antiserum against hepatoma D23 cells was retained (FI 0.75–0.87), whereas that against hepatomas D30 and D33 was lost (FI 0.01–0.13).

Similarly, antisera against the purified hepatoma D23 serum antigen raised in Slonaker rats reacted in a manner similar to the rabbit antiserum (Table 2), so that the unabsorbed antiserum reacted with cell-surface antigens on the three hepatomas used (FI 0.79–0.86). After absorption *in vivo* and *in vitro* with normal Wistar liver membranes, the reactivity against hepatomas D30 and D33 disappeared (FI 0.11–0.20), but that against hepatoma D23 was retained (FI 0.42–0.55).

Table 2 Fluorescence indices of Slonaker anti-D23 purified serum antigen tested against cells from three Wistar tumours

Serum*	Fluorescence index against target cells from: Hepatoma D23	Hepatoma D30	Hepatoma D33
Slonaker anti-D23 purified antigen	0.83, 0.82	0.79, 0.85	0.86, 0.80
Slonaker anti-D23 purified antigen absorbed *in vivo*	0.40, 0.42	0.18, 0.20	0.15, 0.17
Slonaker anti-D23 purified antigen absorbed by normal Wistar rat liver membranes	0.45, 0.55	0.17, 0.11	0.19, 0.20

*Absorption conditions as for Table 1.

The antigen used to immunise rabbits and Slonaker rats was highly purified and homogeneous by all the techniques used (J.G.B. and R.W.B., unpublished). Thus, unless the tumour-specific antigen comprised only a small portion of the immunising material and large amounts of histocompatibility antigens were also present, one must suggest that the hepatoma D23 antigen is a modified histocompatibility antigen.

This hypothesis was examined further by labelling the purified hepatoma D23 antigen with ^{125}I (ref. 12) and examining the binding of this labelled antigen to columns of various immunoabsorbants. Four immunoabsorbants were prepared by coupling to CNBr-activated Sepharose respectively, Slonaker antinormal Wistar liver antiserum, normal Slonaker serum, Wistar antihepatoma D30 antiserum and Wistar antihepatoma D23 antiserum. Each gel (5 ml packed volume in PBS) was packed into large Pasteur pipettes containing a plug of glass fibre to prevent loss of gel beads. ^{125}I-labelled D23 antigen (10 ng in 20 μl PBS) was allowed to run on to each column and the sample was washed in to the column with 0.5 ml PBS. Flow was stopped and the columns incubated at room temperature for 3 h, at which time 10 ml PBS were washed through the column at 0.5 ml min^{-1} and fractions collected. Any bound material was eluted with 3-MCF$_3$COONa *p*H 7.3 and the eluate dialysed against PBS. Both unbound and eluted material were concentrated to 0.5 ml and tested to see if binding to CNBr-Sepharose-linked Wistar antihepatoma D23 antiserum occurred (Table 3).

Of label added to the Slonaker antinormal Wistar liver immunoadsorbent, 75.4% could be bound and eluted and 81.5% of this could be rebound and eluted from Wistar antihepatoma D23. This parallels the results obtained with an initial column of Wistar antihepatoma D23 in that 77.6% of material could be bound and eluted and 87.5% of this could be rebound to a similar column. Conversely, with material added to normal Slonaker or Wistar anti-D30 immunoadsorbants, very little was bound and eluted (29.7 and 25.2% respectively) and this material would not rebind to Wistar antihepatoma D23 (17.5 and 0.7%). Material not bound to these columns (65.4 and 71.6% label), however, would bind to the Wistar antihepatoma D23 (89.5 and 85.75% respectively). Thus the purified hepatoma D23 antigen shows points of identity with antigens recognised by Slonaker rats on normal Wistar liver membranes.

Table 3 Binding of ^{125}I-labelled hepatoma D23 antigen to various immunoabsorbents

Immunoabsorbant	% ^{125}I-labelled hepatoma D23 antigen Unbound	Bound and eluted
Slonaker antinormal Wistar liver	19.5	75.4
Normal Slonaker serum	65.4	29.7
Wistar antihepatoma D30	71.6	25.2
Wistar antihepatoma D23	19.0	77.6
Material unbound on columns of*		
Slonaker antinormal Wistar liver	74.7	20.5
Normal Slonaker	5.4	89.5
Wistar antihepatoma D30	10.8	85.75
Wistar antihepatoma D23	80.0	16.3
Material eluted from column of*		
Slonaker antinormal Wistar liver	15.2	81.5
Normal Slonaker	79.6	17.5
Wistar antihepatoma D30	95.0	0.7
Wistar antihepatoma D23	10.8	87.5

*This material was tested for binding to CNBr-Sepharose-linked Wistar antihepatoma D23.

In this rat hepatoma D23 system, Baldwin and Glaves[10] have shown that when tumour antigens are present there is a deletion of normal antigens recognised by a xenogeneic antiserum. Furthermore, Invernizzi and Parmiani[11] have shown that the tumour-associated transplantation antigens of chemically-induced mouse sarcomas crossreacted with allogeneic histocompatibility antigens. They concluded that in this system the tumour antigen was the result of carcinogen-induced mutation at the level of histocompatibility genes, as there seemed to be a linkage between the tumour antigen and the H-2 system. The work reported here confirms and supports the work of Invernizzi and Parmiani[11] and shows that the tumour-associated transplantation antigen of a chemically induced tumour is a modified histocompatibility antigen.

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Lethality of interferon preparations for newborn mice

WE have previously reported that the daily subcutaneous inoculation of newborn mice with mouse brain interferon preparations was not accompanied by any discernible effect on their growth or development[1]. Far more potent interferon preparations have been shown to inhibit cell division in normal adult mice. Thus, the multiplication of allogeneic spleen and syngeneic bone marrow cells was inhibited when injected into X-irradiated adult mice treated for several days with potent interferon preparations[2], and liver regeneration was inhibited in partially hepatectomised adult mice treated with interferon[3]. We report here that daily injection of potent interferon preparations results in the death of newborn mice. To our knowledge this is the first report of untoward effects associated with interferon treatment of the normal animal. These results may be relevant to the use of interferon in man.

Newborn Swiss or C3H mice from pathogen-free colonies were injected subcutaneously in the interscapular region with the different preparations. They received daily inoculations of 0.05 ml for 1 week beginning on the day of birth and 0.10 ml daily thereafter for 1 month. In all experiments there were at least two litters of mice per group. In some experiments different treatments were undertaken on

Table 1 Lethality of mouse interferon preparations for newborn Swiss or C3H mice

			Injected with:				
Experiment No.	Mouse strain	Type of mouse interferon*	Mouse interferon	Control preparation	Inactivated mouse interferon	Human interferon	Uninoculated
1	Swiss	C243	21/21† (13±1)‡	0/23	NT	NT	0/27
2	Swiss	C243	23/23 (11±1)	0/11	NT	NT	0/22
3	Swiss	C243	22/22 (13±2)	NT	NT	NT	0/21
4	Swiss	C243	26/26 (9±1)	0/18	NT	0/23**	0/53
5	Swiss	C243	15/15 (10±1)	NT	0/15‖	NT	0/19
6	C$_3$H	C243	15/15 (9±1)	0/30	0/15¶	NT	0/18
7	Swiss	L§	6/7 (14±1)	NT	NT	NT	0/18
Total:			128/129	0/82	0/30	0/23	0/178

*A different mouse C243 cell interferon preparation (titre 1 : 800,000) was used in each of experiments 1–6.

†Number of mice dead/total number of mice injected, based on survival at 1 month. Newborn mice dying in the first 2 d of life are not included.

‡Mean day of death ± s.d.

§Titre of the Sendai virus-induced L cell interferon was 1 : 240,000.

‖Inactivated by heating at 60 °C for 1 h; residual titre 1 : 100,000.

¶Inactivated by treatment with 0.02 M sodium periodate at pH 4 for 3 h at 4 °C; residual titre 1 : 600.

**Titre of human leukocyte interferon was 1 : 2,000,000 (MRC reference interferon (69/19) units) as assayed on human diploid fibroblasts.

NT, Not tested.

Mouse interferon from suspension cultures of C243 cells[4] induced with Newcastle Disease Virus (NDV) was concentrated and semipurified as described previously[5]. Mock interferon was prepared in a manner identical to that used in the preparation of interferon with the exception that the interferon inducer, NDV, was either omitted altogether, or added for 1 h immediately before collecting the culture supernatant (experiment 4). For experiment 7, mouse interferon prepared in monolayer cultures of L cells inoculated with Sendai virus, was concentrated by selective precipitation with ammonium sulphate as described for the C243 cell interferon[5]. All interferon and control preparations were further dialysed for 24 h at 4 °C against 0.01 M perchloric acid before testing for toxicity on a line of L1210 cells resistant to interferon[6].

Specific activity of mouse C243 cell interferon preparations was in the range 0.7–3×10^6 units per mg protein and that of the L cell interferon was 2×10^4 units per mg protein. C243 cell control preparations contained approximately the same amount of protein per ml as the C243 cell interferon (that is, 1.5–3.4 mg ml^{-1}). Partially purified human interferon prepared in leukocyte suspensions inoculated with Sendai virus was generously provided by Dr K. Cantell[7]. Specific activity was 3.5×10^5 units per mg protein. Interferon units quoted are as measured in our laboratory and one of our units equals 4 mouse interferon reference units.

labelled mice within the same litter. All mice were weighed daily. On occasions moribund mice were killed for histological examination of their organs. Some mice in the different groups surviving at 1 month were killed, others were observed for 3–6 months.

Newborn mice inoculated with interferon (titre 1:800,000) seemed to develop and grow normally for the first 6–7 d. In the ensuing few days an increasing difference in weight was observed between interferon-treated mice and their control littermates. Interferon-treated mice died between day 8 and 14. The lethality of mouse interferon preparations for newborn mice is illustrated in Table 1 and the salient points may be summarised as follows: All (122 out of 122) newborn Swiss and C3H mice inoculated daily with C243 cell interferon preparations (titre 1 : 800,000 died. Six out of seven newborn Swiss mice inoculated with another type of mouse interferon preparation (titre 1:240,000), derived from L cells (and induced with a different virus), also died. No deaths were observed in mice inoculated daily with control preparations (0 out of 82); with inactivated mouse interferon preparations (0 out of 30); with human leukocyte interferon preparations (0 out of 23) or left uninoculated (0 out of 178).

To determine the minimal lethal amount of interferon, newborn Swiss mice were inoculated daily with tenfold dilutions of an interferon preparation (titre 1:800,000). Only mice inoculated with the undiluted interferon preparation died, whereas those inoculated with preparations of titre 1 : 80,000 or less seemed to grow normally; and no abnormalities were observed on histological examination of their organs at 1 month. These findings are in accord with our previous results[1] indicating that newborn mice inoculated with mouse brain interferon preparations (titre 1 : 8,000–1 : 16,000) developed and grew normally without presenting any abnormalities on histological examination. In experiment 5 (Table 1) mouse interferon was only partially inactivated by heating and the residual titre was found to be 1:100,000. Mice treated with these preparations seemed to develop normally. Thus, our results suggest that daily inoculation of newborn mice with interferon preparations of titre 1 : 100,000–1 : 240,000 (experiment 7, Table 1) would prove fatal for 50% of the mice.

The most striking abnormality on killing moribund interferon (both C243 and L cell interferon) treated mice was a pale grey liver. Examination of the liver of these mice under the light microscope revealed large areas of cell degeneration and necrosis without any inflammatory reaction (Fig. 1*c* and *d*). Examination of liver sections under the electron microscope showed an accumulation of fat, decreased glycogen, swelling of both the mitochondria and the rough endoplasmic reticulum, and discrete nuclear and nucleolar abnormalities. No viral particles were observed. The only other abnormalities on light microscopic examination were: some diminution of the cortical region of the thymus in some mice (and presence of some pycnotic cells in the cortex) and poorly differentiated germinal follicles in the spleens of most mice.

In the experiments described, interferon treatment of newborn mice proved lethal when begun on the day of birth and continued daily. When, however, treatment was initiated on day 6 of life and continued daily for 14 d, mice seemed to grow normally and no mortality was observed. Two mice were killed on day 21 and histological examination of their livers revealed an increased granularity of the cytoplasm without cellular degeneration. The resistance of these mice may be related to the stage of differentiation of the liver at the time interferon inoculations were begun.

We have no reason to believe that the interferon preparations exerted a nonspecific toxic effect, or that an infectious agent was responsible for the hepatic lesions observed in interferon-treated mice. The results of pertinent experiments may be summarised as follows: First, all interferon and control preparations were incubated (1 : 10 final dilution, that is, 80,000 interferon units) with interferon-resistant L1210 cells[6]. Neither the multiplication nor the viability of these cells was affected over a 48-h period. Representative interferon preparations exerted no detectable toxic effect on confluent monolayer cultures of mouse L or chick embryo cells. Second, one litter of 10 newborn Swiss mice was inoculated intracerebrally daily for 3 d with a C243 cell interferon (titre 1:800,000). All mice survived. No toxic effects were noted and no abnormalities were observed over a 3-month period. Third, two moribund interferon-treated mice with gross liver abnormalities (confirmed

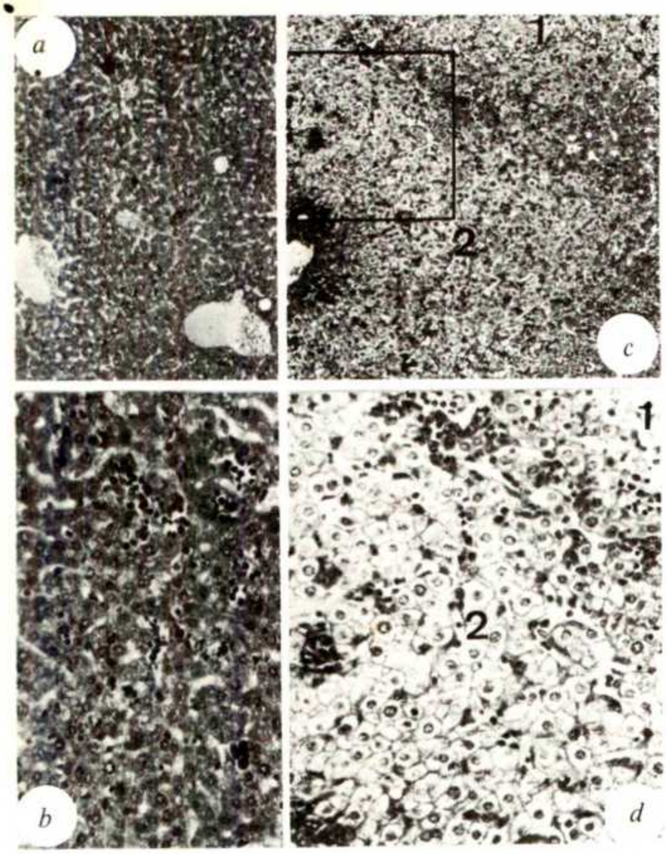

Fig. 1 Sections of liver of 10-d-old Swiss mice. *a* and *b*, Untreated (× 75.6 and × 189); *c* and *d*, treated with a C243 interferon preparation (titre 1:800,000; × 75.6 and × 189) showing focal area of necrosis (1) and diffuse cellular degeneration (2).

on light and electron microscopic examination) were killed and extracts of liver and kidney were inoculated subcutaneously into newborn mice and into primary cultures of newborn mouse kidney. No abnormalities were observed in newborn mice kept for 3 months. No cytopathic agent was detected in cell culture. Using immunofluorescent techniques, inoculated cell cultures did not seem to contain either lymphocytic choriomeningitis or murine hepatitis viral antigens.

Our results show therefore that daily inoculation of newborn mice with potent interferon preparations is lethal and that mice die with extensive liver damage. Our findings may be relevant to the treatment of patients with exogenous interferon. If we suppose that a newborn baby is as sensitive to human interferon preparations as a newborn mouse is to mouse interferon, then our results suggest that the daily inoculation of 50 ml human interferon preparation (titre 1 : 800,000) might represent a lethal dose for a 3-kg baby (that is, compared with 0.05 ml for a 3-g mouse). Our results also raise the possibility that lower doses of interferon may cause adverse effects in newborn infants that are not immediately apparent. We are aware of the hazard of this analogy, but patients are currently being treated with potent interferon preparations and it is therefore incumbent on clinicians to be aware of the potential dangers of interferon therapy at least in infants. On the other hand it is also important to emphasise that adult patients have been treated twice or three times weekly for extended periods of time with potent leukocyte interferon preparations without showing any untoward effects[8,9].

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Effect of cortisol, prolactin and ADH on the amniotic membrane

VERY little is known about the endocrine regulation of amniotic fluid volume and composition[1,2]. Vizsolyi and Perks[2] have described a technique for studying the transfer of fluid across the amniotic membrane of the guinea pig *in vitro*. They showed that arginine vasotocin and arginine vasopressin (antidiuretic hormone, ADH) could stimulate the movement of fluid from the maternal to the foetal side of the membrane. Because prolactin is found in remarkably high concentrations (up to 10 µg ml^{-1}) in primate amniotic fluid[3,4] and affects renal function[5–8], we have investigated the effect of prolactin as well as of arginine vasopressin on the guinea pig amniotic membrane. Because cortisol can reverse the usual antidiuretic effect of ADH and because prolactin can restore this effect[9], we have also investigated the action of cortisol. We have shown that ADH and prolactin have opposite effects on the amniotic membrane and that, in both cases, their actions can be reversed by cortisol. We suggest that fluid transfer across the amniotic membrane may provide an insight to the functioning of the kidneys, the ciliary body and the choroid plexus.

The apparatus used was similar to that described by Vizsolyi and Perks[2]. Female guinea pigs were killed in the last three weeks of pregnancy. Two portions of the amniotic membrane were tied over the ends of two glass tubes, each with an end area of 1 cm^2; the foetal surfaces of the membrane faced into the tubes. The tubes were filled to a depth of 10 cm with 'amniotic' saline (Fig. 1) and suspended in oxygenated baths of 'maternal' saline with the fluid level in the tubes 2 cm above that in the bath. The tubes did not float, but were supported by balanced levers connected to rods passing through Sangamo Weston displacement transducers. Changes in weight of the tubes resulting from fluid movements produced movements of the levers and changes in transducer output which were continuously recorded using Devices equipment. The system was calibrated by adding known volumes of fluid to the tubes. Both the relative osmolarities and the 2-cm H_2O pressure head favoured movements of fluid from the tube ('foetal' side) to the bath ('maternal' side). Eight animals were used in preliminary experiments in which various hormone concentrations were tested. Two sets of six experiments were then carried out as indicated in Table 1; in each experiment, the *A* and *B* membranes came from the same animal. The results are shown in Figs 1 and 2. All hormones were added to the foetal side of the membrane.

In the control period no significant movement of fluid

Table 1 Experimental addition of hormones to the foetal side of the amniotic membrane *in vitro*

Experiment	Periods* 1	2	3	4
1*A*	Control	Prolactin (200 ng ml^{-1})	Prolactin (1,000 ng ml^{-1})	Prolactin (1,000 ng ml^{-1})+ cortisol (200 ng ml^{-1})
1*B*	Control	ADH (0.05 IU ml^{-1})	ADH (0.1 IU ml^{-1})	ADH (0.1 IU ml^{-1})+ cortisol (200 ng ml^{-1})
2*A*	Control	Cortisol (200 ng ml^{-1})	Cortisol+ADH (0.05 IU ml^{-1})	
2*B*	Control	Cortisol (200 ng ml^{-1})	Cortisol+prolactin (1,000 ng ml^{-1})	

* Each period lasted for 30 min.

occurred in either direction Prolactin at 200 ng ml^{-1} caused a transfer of fluid to the maternal side; 1,000 ng ml^{-1} caused a further movement in the same direction. The addition of cortisol (200 ng ml^{-1}) to the prolactin completely reversed the direction of fluid movement The opposite was found with ADH (0.05 and 0.1 IU ml^{-1}); it caused a movement to the foetal side which was reversed by cortisol. In the second series of experiments, cortisol added alone caused a fluid movement to the maternal side. ADH then caused a further movement in the same direction but prolactin had the reverse effect, moving fluid to the foetal side.

With respect to the amniotic fluid, the prolactin was used in concentrations which are, in primates at least, physiological[3] whereas the other two hormones were probably at concentrations much greater than those found in amniotic fluid[2,10]. We therefore suggest that the effect of prolactin alone is likely to be the one which is of practical importance If prolactin does cause a movement of fluid out of the amniotic sac this could account for the hydramnios which is frequently found when human amniotic fluid prolactin concentrations are low[3].

It is already known that prolactin, cortisol and ADH can interact in the formation of urine[7-9] and of aqueous humour[11]. Cortisol and prolactin have been shown to have opposite effects on some fish bladders[12,13]. Because of its relative simplicity, the guinea pig amniotic membrane may provide a good mammalian model for our understanding of amniotic fluid regulation and as an indication as to what may be happening at other sites where urine, aqueous humour, cerebrospinal fluid and gastrointestinal secretions are formed.

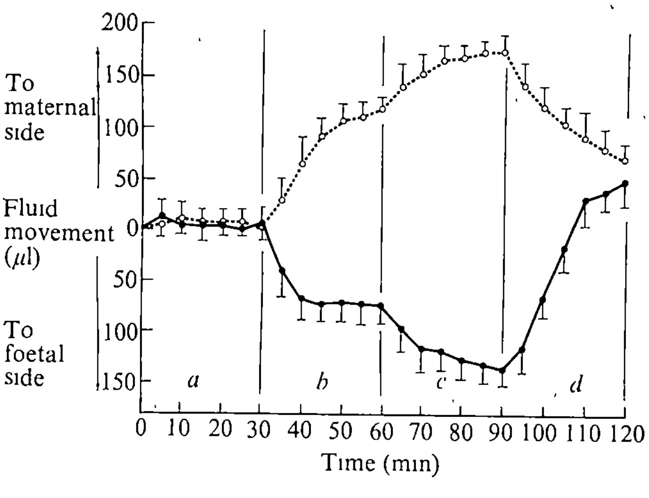

Fig. 1 The fluid movements during the various incubation periods in experiments 1*A* and 1*B* (Table 1) At each point the net amount of fluid which has moved since time zero is indicated. Each figure represents the mean of six experiments and the bars indicate the s.e.m The 'amniotic' saline had the following composition: sodium 125.0, potassium 6 2, calcium 4.5 and magnesium 2.3 mM l^{-1}, all added as chloride· 1 0 g l^{-1} glucose: 10 ml phosphate buffer at *p*H 7.8 was added per litre, giving a final *p*H of 7.4 and osmolarity of 286 mosmol l^{-1}. The 'maternal' saline was made up as follows: sodium 150.0, potassium, 5.5, calcium 4 4, magnesium 2.6 mM l^{-1}, all added as for the amniotic saline giving a final *p*H of 7.4 and osmolarity of 314 mosmol l^{-1} The maternal saline was bubbled continuously with oxygen and maintained at 37 °C. Vizsolyi and Perks[2] studied the compositions of guinea pig amniotic fluid and maternal plasma and devised the 'amniotic' and 'maternal' salines to reproduce the natural fluids as closely as possible *a*, Control; *b*, prolactin (200 ng ml^{-1}), ADH, 0 05 IU ml^{-1}; *c*, prolactin (1,000 ng ml^{-1}), ADH, (0 1 IU ml^{-1}); *d*, prolactin (1,000 ng ml^{-1}) plus cortisol (200 ng ml^{-1}), ADH, (0.1 IU ml^{-1}) plus cortisol (200 ng ml^{-1}) ○, Prolactin; ●, ADH.

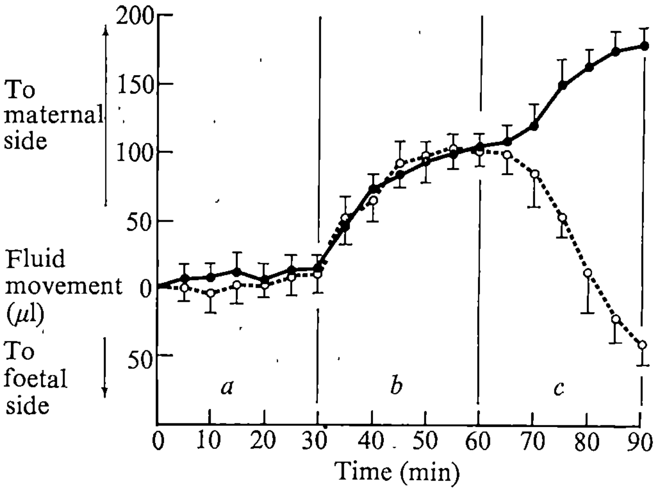

Fig. 2 The fluid movements during the various incubation periods in experiments 2*A* and 2*B*. *a*, Control; *b*, cortisol (200 ng ml^{-1}); *c*, cortisol (200 ng ml^{-1}) plus ADH (0.05 IU ml^{-1}) or prolactin (1,000 ng ml^{-1}). Each point represents the mean of six experiments and the bars indicate s.e.m. ●, ADH, ○, Prolactin.

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Action of progesterone on preoptic thermosensitive neurones

FEMALE body temperature rises by about 0.5 °C at or near ovulation and remains elevated during the luteal phase of the menstrual cycle. Sustained elevation of basal body temperature is observed also during early pregnancy. These effects have been attributed to progesterone, but little is known about the mechanisms involved[1]. We have now found that the hormone affects the activity of the thermosensitive neurones in the preoptic area of the brain.

These neurones change their discharge frequency in response to changes in local brain temperature: that of warm-sensitive neurones increases with a rise in temperature[2–3], while that of cold-sensitive neurones increases with a fall in temperature[4]. Intravenous pyrogen in anaesthetised animals (cat and rabbit) decreases the activity of warm-sensitive neurones and increases that of cold-sensitive neurones[5–8].

Our investigation followed preliminary studies of 23 unanaesthetised female rabbits, injected intravenously with progesterone (more than 5 mg kg^{-1}). There was a rise in rectal temperature of 0.6 °C, with the highest elevation in core temperature 2 h after injection. Progesterone was dissolved in propylene glycol, but the solvent alone did not cause temperature to rise. We have therefore investigated the effect of systemic administration of progesterone on the thermosensitive neurones.

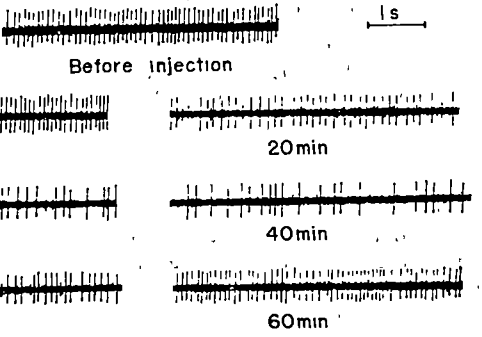

Fig. 1 Discharges of the preoptic warm-sensitive neurone in response to intravenous administration of progesterone in the female rabbit.

Thirty-four female rabbits (2.5–4.0 kg) were anaesthetised with urethane (1 g kg^{-1}), tracheotomised and mounted on a stereotaxic apparatus. (Details of the method have been described before[3].) A thermode was implanted in the preoptic area, 1.5 mm lateral to the midline, and brain temperature was measured by a thermocouple placed in the frontal plane, 3.0 mm from the thermode. Impulses of single neurones were recorded on tape and impulses per second were counted with a data processing computer. A small amount of iron was then deposited electrolytically at the tip of the electrode for histological localisation. A neurone with a regular rate of firing was identified as warm-sensitive, cold-sensitive or temperature-insensitive by its responses to local warming and cooling. The effect of progesterone was observed on twenty warm-sensitive, seven cold-sensitive and five insensitive neurones. Progesterone was dissolved in 50% propylene glycol (5 mg ml^{-1}). When the thermal response of a neurone had been observed, the brain temperature was kept at 37.5 °C and progesterone (10 mg in 2 ml) was injected intravenously. In a typical warm-sensitive neurone (Fig. 1), the average rate of firing before injection was 11.2 impulses s^{-1}. After a latency of about 20 min, this decreased to 7.7 at 24 min, to 6.6 at 40 min and returned to 10.0 at 60 min. Of the 20 warm-sensitive neurones tested, 15 decreased their firing rate by 38–70% after administration of progesterone. The thermal responses of these 15 neurones had not changed when observed 60 min after administration, with the exception of one neurone which showed a decreased thermosensitivity. The rate of firing of five warm-sensitive neurones was unchanged for 2 h after intramuscular administration of progesterone.

In seven cold-sensitive neurones the rate of firing increased after administration of progesterone given intramuscularly in three cases and intravenously in the other two. In one of these neurones, the rate of firing increased from 6.4 to 12.0 impulses s^{-1} 6 min after injection. Five thermally-insensitive neurones were not affected by progesterone and two warm-sensitive neurones were not affected by propylene glycol.

Plasma concentration of progesterone rises quickly after intravenous administration and returns to a control level in about 1 h (refs 9 and 10). This may explain the short duration of action of progesterone that we observed. We do not know whether the thermosensitive neurones tested in this study are true thermoreceptors or interneurones transmitting temperature signals in a complex neural network of temperature regulation. Nor are we certain whether the neuronal effects which we observed were caused by progesterone *per se* or by its metabolites. Furthermore, the quantitative relationship between oestrogen and progesterone in the luteal phase is complex and oestrogenic hormones are known to cause a slight decrease in body temperature[11–12].

It seems likely, however, that the decreased activity of warm-sensitive neurones and the increased activity of cold-sensitive neurones in the preoptic area result in an upward shift of the 'set point' of body temperature, as with the effects of pyrogen. The rise of basal body temperature in the luteal phase seems to be related, at least in part, to the direct or indirect action of progesterone on preoptic thermosensitive neurones.

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Excitation of phasically firing supraoptic neurones during vasopressin release

THE neurohypophysial hormones vasopressin and oxytocin are synthesised within separate neurones of the hypothalamic supraoptic and paraventricular nuclei[1] and each hormone can be liberated independently. Haemorrhage[2] and bilateral

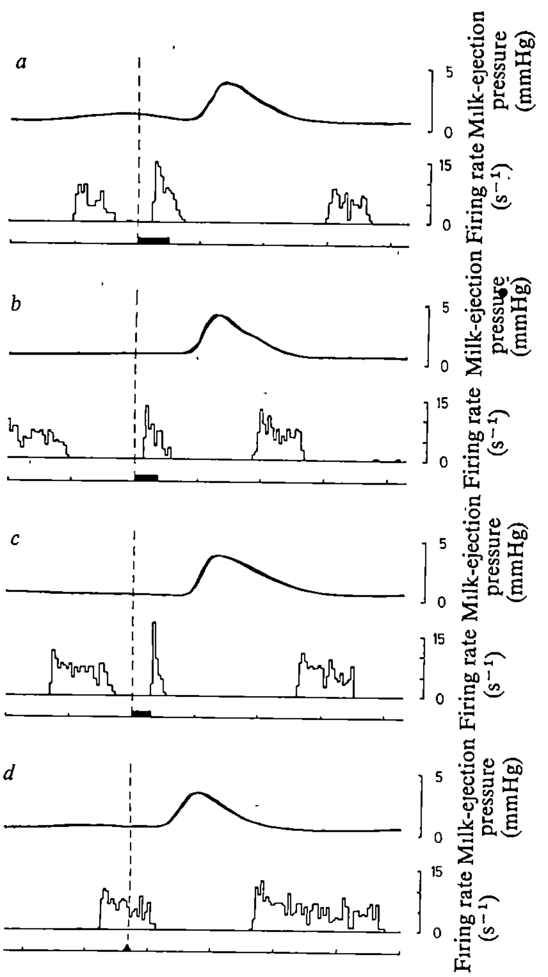


Fig. 1 Responses of a phasically active supraoptic neurone and of intramammary pressure to bilateral carotid occlusion. Antidromically identified neurone fired with bursts of activity separated by silent intervals. Bilateral occlusion of the common carotid arteries (black bars) for 15 s (*a*), 11 s (*b*) and 9 s (*c*) evoked a short supplementary burst of activity from the neurone 4–9 s after the onset of occlusion (dashed line). In two instances (*a* and *c*) the peak firing of the evoked burst exceeded that of the spontaneously occurring bursts. The rise in intramammary pressure occurred 18–20 s after the onset of each occlusion, and was of similar amplitude and duration to that occurring 16 s after the intravenous injection of 250 μU arginine vasopressin in the black triangle in (*d*). Time base, 30 s.

carotid occlusion[3] release vasopressin without oxytocin, whereas oxytocin is released without vasopressin during suckling[4,5] and parturition[6]. The neuroendocrine cells produce all-or-none action potentials which propagate to the neural lobe, where they trigger hormone secretion[7]. Action potential firing of individual endocrine cells falls into two principal categories: most fire continuously and randomly at 1–2 spikes s^{-1}, while the rest have a phasic pattern of firing, periods of bursting activity alternating every 10–60 s with periods of silence[8].

Lincoln and Wakerley[9] recorded the firing rates of supraoptic neurones in rats during suckling, and found that the reflex excited predominantly the randomly firing neurones. We have therefore investigated supraoptic neuronal activity during stimulation which is known to release only vasopressin. We found that, during bilateral occlusion of the common carotid arteries, supraoptic neurones are excited and that this activation is confined almost exclusively to phasically active neurones.

We used lactating female rats under urethane anaesthesia (1.2 mg kg^{-1}). Their common carotid arteries were occluded by a clamp which exerted no tension. Blood pressure and intramammary pressure were measured routinely. The brain was exposed from the ventral surface and supraoptic neurones were identified by antidromic invasion after electrical stimulation of the pituitary stalk[10].

Of 101 cells studied, 60 had a random pattern of firing, 33 had a phasic pattern and 8 were silent. Bilateral occlusion of the common carotid arteries reproducibly excited 30 of the phasically firing neurones. Occlusion of the arteries during the silent period of a cycle was followed by a burst of action potentials which started less than 10 s after the onset of occlusion. If applied during a spontaneous burst, occlusion often prolonged that burst. Random neurones responded to occlusion in a much more varied manner: 4 were excited, 14 showed slight excitation followed by prolonged inhibition, 24 showed inhibition alone and 18 were unaffected. Of the silent cells, 4 were excited.

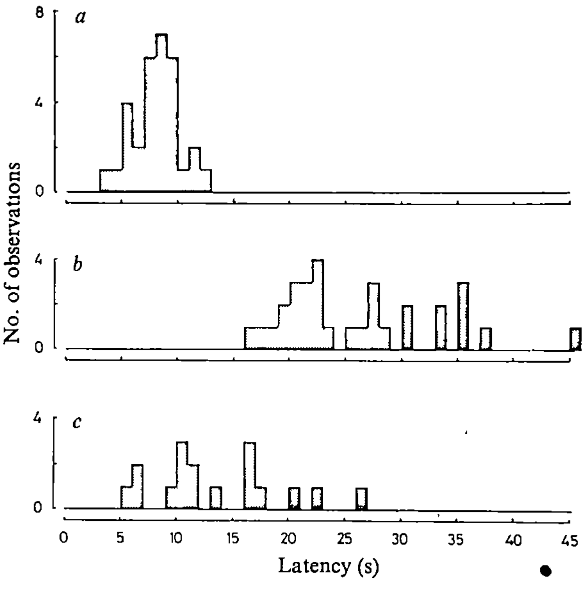
The response of a phasic neurone in a lactating rat to carotid occlusions of various duration is illustrated in Fig. 1. The neurone responded to periods of occlusion of 15, 11 and 9 s with a burst of activity starting 4–9 s after the onset of occlusion.

In Fig. 1*a* and *c* the evoked bursts had a higher peak rate than the spontaneous bursts. Approximately 20 s after the onset of neuronal firing, intramammary pressure increased, showing that one or both neurohypophysial hormones had been released. The increase in pressure was approximately equivalent to that which followed the injection of 250 μU arginine vasopressin into a jugular vein. In this neurone the duration of the evoked burst seemed to be controlled by the duration of the occlusion; this was also seen in some other neurones.

Recording of intramammary pressure in lactating rats takes advantage of the fact that vasopressin causes milk ejection even though its activity is only about 20% of that shown by oxytocin. If the milk-ejection response to carotid occlusion was caused by hormone released as a result of activation of supraoptic neurones, the response should begin approximately one circulation time after the onset of the evoked burst of activity. This occurred regularly, as shown in Fig. 2

The mean latency of the milk-ejection response was 26.2 s. This must be explained by the delay before the cells are activated and the time taken by the hormone to reach the mammary

Fig. 2 Histogram of the latencies of neuronal and milk-ejection response to bilateral carotid occlusion and to exogenous vasopressin. Results from eight supraoptic neurones, *a*, Latencies between the beginning of occlusion and the onset of the stimulus-evoked burst of action potentials ranged between 3 and 13 s, with mean latency (±s.e.) of 8.1±0.4 s (n = 31). *b*, Latencies between the beginning of the same occlusions as in *a* and the onset of the stimulus-evoked milk-ejection response. Mean latency (±s.e.) was 26.2±1.3 s (n = 31). *c*, Latencies between the injection into the jugular vein of 0.25–1.0 mU vasopressin and the onset of the ensuing milk-ejection responses. Mean (±s.e.), 13.7±1.4 s (n = 17).



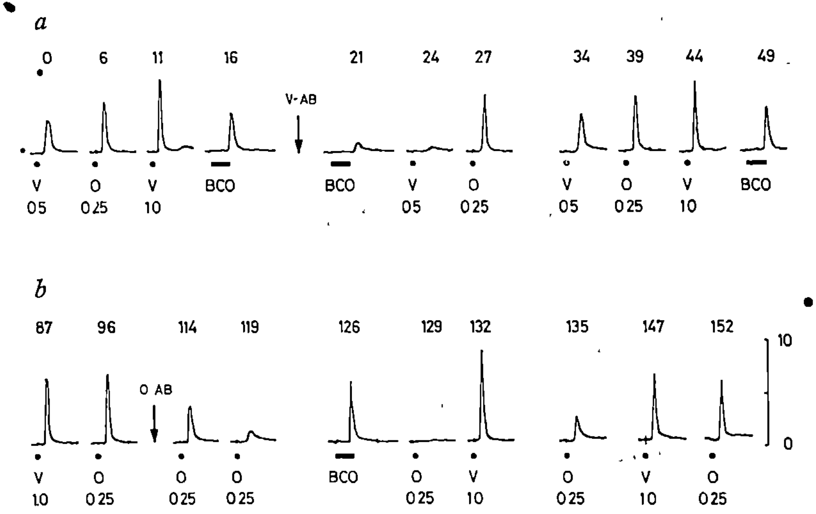


Fig. 3 Effects of injected antibodies to vasopressin and oxytocin on milk-ejection responses to bilateral carotid occlusion and to exogenous neurohormones. Records show milk-ejection responses after exogenous vasopressin (V), exogenous oxytocin (O) and 30 s bilateral carotid occlusion (BCO) before and after intravenous injection into the jugular vein of antibodies at the arrows. Numbers beneath records give concentrations of hormones in mU. Vertical scale is in mmHg. Figures above the responses indicate time (min) after the start of the experiment. Injections or occlusions were made at approximately 5-min intervals, except immediately after antibody injection. The responses are consecutive, but the record is not continuous. *a*, Effect of vasopressin antibodies (V-AB). BCO caused milk-ejection responses equivalent to 0.5 mU vasopressin and slightly less than 0.25 mU oxytocin. After injection of antibodies, responses to BCO and vasopressin are almost abolished whereas response to oxytocin is unaffected. *b*, Effect of oxytocin antibodies (O-AB). Injection of antibodies at arrow progressively abolishes response to 0.25 mU oxytocin without reducing responses to either BCO or vasopressin. Note reversibility of the effects of both antibodies.

gland after release. The observed latency of the onset of the cell response was 8.1 s, and the observed latency of milk ejection following injection of vasopressin into a jugular vein was 13.7 s. The difference between the observed latency of the milk-ejection response to occlusion and the sum of the latter two figures is because the cells do not reach their peak firing rate immediately and because injections into the jugular vein underestimate the time taken by hormone to reach the mammary gland from the pituitary.

There is evidence that carotid occlusion liberates only vasopressin in cat and dog[11], but there is no published evidence that this is so in rats. Since both neurohypophysial hormones increase intramammary pressure, it was necessary to establish that the responses we observed were due to vasopressin and not to oxytocin. We used two approaches. First, rats anaesthetised by methane were subjected to a 60-s occlusion and then decapitated. The extracted trunk plasma[12] was assayed by radioimmunoassay for vasopressin and oxytocin[13] and compared with that of extracts from rats subjected to the same surgery but omitting carotid occlusion. The results showed that occlusion increased mean (±s.e.) plasma vasopressin from 303 ± 70 to 970 ± 199 pg ml^{-1} ($n = 8$; t test, $P < 0.01$) but the increase in oxytocin was much smaller and not significant ($P > 0.1$). Second, intrajugular injections of the anti-vasopressin and the anti-oxytocin antibodies were given to lactating rats in conjunction with bilateral carotid occlusion. Results from one animal are shown in Fig. 3. Injection of 0.2 ml of undiluted rabbit plasma containing the specific anti-vasopressin antibody[13] depressed the milk-ejection responses to carotid occlusion and to intravenously injected arginine-vasopressin, without affecting that to exogenous oxytocin. Conversely the oxytocin antibody abolished the response to oxytocin but to neither occlusion nor vasopressin.

Occlusions of 5–10 min duration cause vasopressin secretion in cats and dogs, and following section of the sinus nerves, hormone secretion is abolished, indicating that the response is mediated neurally[11]. Our results indicate that in the rat occlusion of much shorter duration is a stimulus for vasopressin secretion and excites nearly all phasically firing supraoptic neurones; most randomly firing cells are either inhibited or unaffected.

These observations complement the report that, during naturally occurring milk ejection in rats, about half of the randomly firing supraoptic neurones were powerfully excited, whereas phasic neurones were not involved[9]. These findings suggested that randomly firing neurones are those that secrete oxytocin. Since the excitatory input to the supraoptic nuclei in response to bilateral carotid occlusion is apparently channelled almost exclusively to the phasically active neurones, these cells might represent a distinctive class of vasopressin-secreting neurones.

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Activity of phasic neurosecretory cells during haemorrhage

Approximately 25% of the neurones projecting to the neurohypophysis from the paraventricular (PV) and supraoptic (SO) nuclei of the hypothalamus fire phasically and such a pattern is seldom seen in other hypothalamic cells[1]. Phasic neurones respond to osmotic stimuli[2] but are rarely activated by suckling[1,3]. It is tempting therefore, to associate these cells with the secretion of vasopressin rather than oxytocin. The functional relationship of their phasic pattern to hormone release, however, remains obscure. We have studied phasic PV neurones during haemorrhage, which releases vasopressin, sometimes with small amounts of oxytocin[4]. Haemorrhage evoked changes in phasic units over a period long enough for a detailed analysis of their behaviour. Our results show the importance of pattern

Table 1 Changes in the activity quotient and intraburst frequency of phasic neurosecretory cells during blood removal and blood replacement

	Control values	Change during blood removal				Change during blood replacement			
Time from start (min)		1	2	3	4	1	2	3	4
Activity quotient	0.52	+0.09*	+0.14*	+0.15†	+0.24†	−0.25*	−0.29†	−0.47†	−0.24*
(Mean ±s.e.)	(±0.06)	(±0.04)	(±0.06)	(±0.05)	(±0.07)	(±0.10)	(±0.06)	(±0.09)	(±0.16)
Intraburst frequency‡ (spikes s^{-1})	7.59	+0.39	+0.49	+2.20*	+5.10*				
(Mean ±s.e.)	(±1.50)	(±0.29)	(±0.37)	(±0.71)	(±2.13)				

*$P < 0.05$. †$P < 0.01$. Significance levels were determined by the paired t test.

‡ Many of the cells were quiescent during blood replacement and this precluded a statistical analysis of the changes in intraburst frequency during this period.

modulation in phasic neurones and suggest that the mechanism for vasopressin release differs from that described for oxytocin release during suckling[1,3].

Lactating female rats (300–350 g), anaesthetised with urethane (1.1 g kg^{-1}), were prepared for intramammary pressure recording[1]. Cannulae were introduced into the saphenous artery for monitoring blood pressure and into the right atrium through the jugular vein to remove blood. Animals were then placed in a stereotaxic frame and antidromically identified units were recorded in the PV nucleus by techniques described previously[1,3]. After locating a phasic unit, we recorded 10–15 min of baseline activity before examining the effect of a 4-ml haemorrhage over a period of 4 min (1.3 ml per 100 g body weight; 15% of blood volume). The first and last 0.5 ml of blood collected were used for plasma osmotic pressure determination and vasopressin assay. Two minutes after haemorrhage, the blood was returned over 4–6 min. The volume of blood taken for assay was replaced with warm isotonic saline. Where possible, the test was repeated for each cell 20–30 min later. Samples were centrifuged and the plasma stored frozen at −20 °C until assay. The blood cells were washed in saline and reinjected in a minimum volume of isotonic saline before a further unit was tested.

Vasopressin could not be detected in all samples after haemorrhage, probably because of the relative insensitivity of the assay (rat antidiuretic assay: minimum detectable level > 32 μU per ml plasma). In a series of tests, however, the plasma content of antidiuretic activity after blood removal (median 56 μU ml^{-1}, $n=21$) was significantly higher ($P < 0.001$, Mann–Whitney U test) than control levels (median < 32 μU ml^{-1}, $n=32$). The median value for plasma oxytocin was < 25 μU ml^{-1} both before ($n=23$) and after ($n=17$) haemorrhage, but in two tests oxytocin release was detected. Blood pressure usually dropped from about 100 mmHg to 30–40 mmHg during blood withdrawal and returned to normal levels after blood replacement (Fig. 1). Plasma osmotic pressure (range 295–315 mosmol kg^{-1}) did not change.

Sixty-nine antidromically identified PV units were encountered, of which 15 (22%) fired in a phasic pattern. At histological examination, all were shown to be located in the PV nucleus. The mean duration of the bursts varied considerably between cells (range 6.9–48.6 s) as did the duration of the silent periods (range 9.9–22.2 s); however, for individual neurones the periodicity was quite regular. Ten of the phasic units were recorded throughout the full haemorrhage test. For each minute of the different periods (control, blood out and blood back) the following parameters of spike activity were measured: (1) The activity quotient, Q, which is the total duration of the bursts (s) in 1 min divided by 60; (2) the intraburst frequency, f, representing the mean firing rate within the bursts; and (3) the overall frequency, F, which is the total number of spikes in 1 min divided by 60.

During haemorrhage, the most consistent effect was a prolongation of the bursts (Fig. 1) resulting in a significant increase in Q during each of the first 4 min (Table 1). In

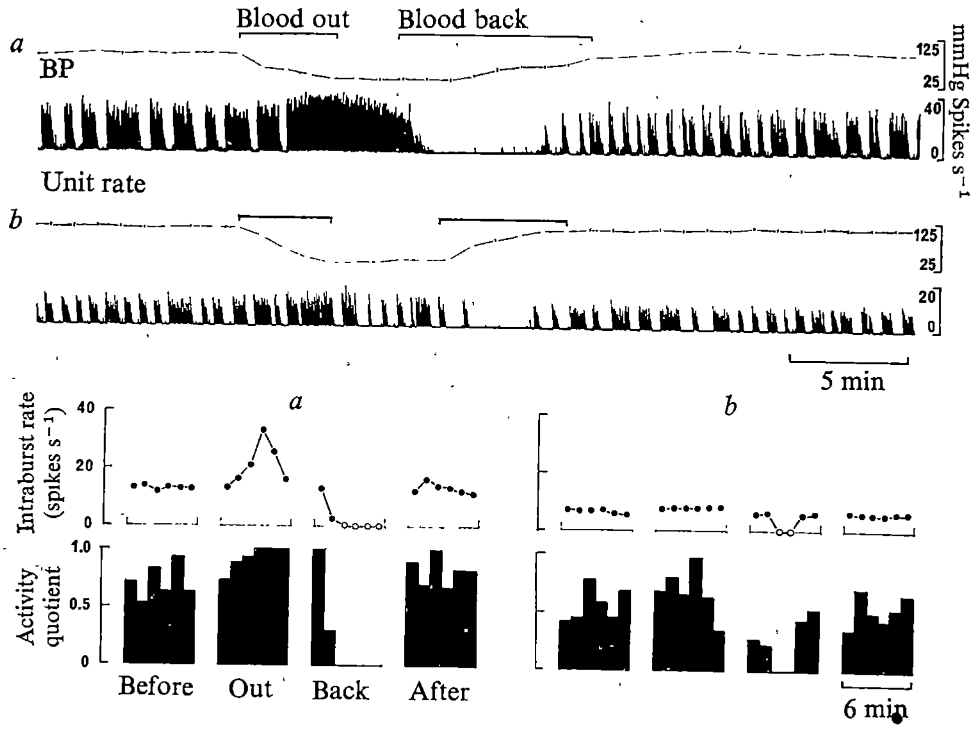

Fig. 1 Records of the firing pattern of 2 phasic cells (a and b) to show the changes which occurred during the withdrawal and replacement of 4 ml blood. Blood pressure decreased rapidly during blood withdrawal and returned to control level soon after blood replacement. Beneath the records are plotted the changes in firing rate within the bursts (f) and the proportion of time for which the cells were firing (activity quotient, Q). Haemorrhage increased Q for each of the cells tested and one of the cells showed an increase in f. In contrast, blood replacement decreased the values of both Q and f. ○, Period during which the cells were totally inactive.

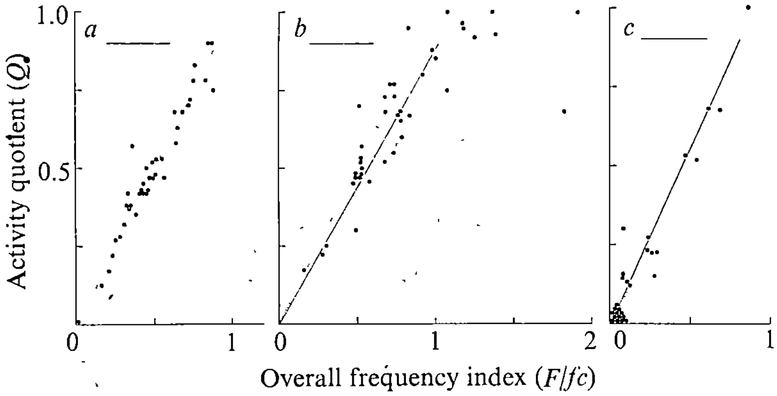

Fig. 2 The relationship between activity quotient (Q) frequency within the bursts (f) and overall frequency (F) during the control period (*a*), blood withdrawal (*b*), and blood replacement (*c*). Q, f and F have been established for each response and they are related by the equations $F = Qf$ or $Q = F/f$. So that the results of all the tests could be considered as a whole, we used the expression F/fc where fc is the mean frequency within the bursts during the control period. If the intraburst frequency remains constant (that is, equal to fc), Q and F/fc are related linearly and all the points fall on the line defined by $Q = F/fc$. If f increases the points fall on the right of the line; if f decreases the points fall on the left of the line. Regression lines have been calculated for the three experimental conditions. For values of Q between 0 and 0.90, the correlation coefficients are highly significant (respectively $r = 0.97$, $r = 0.68$, $r = 0.97$ and $P < 0.001$) and the regression coefficients of the three lines are not significantly different ($P < 0.3$ variance ratio test and Student's t test). $F/fc = 1.01Q - 0.01$ (*a*); $1.06Q + 0.07$ (*b*); $0.9Q + 0.01$ (*c*)

extreme cases, this remained at unity for several minutes (cell *a*, Fig. 1). Intraburst frequency (f) also changed but less consistently and later: the mean increase was significant during the third and fourth minutes. These events resulted in an increased mean overall frequency (F) from a control level of 3.93 spikes s^{-1} to 9.60 spikes s^{-1} in the fourth minute of haemorrhage. Blood replacement produced opposite effects, with reduction in Q resulting from shortened bursts. Many neurones became silent, but occasionally when bursts did occur f decreased. During the third minute F fell to 0.1 spikes s^{-1}. Recovery occurred within a few minutes of the end of the test. In neurones tested twice, the second response was similar to the first.

The relative importance of the change in burst duration and in intraburst frequency is shown in Fig 2, which correlates Q with a measure of the change in overall frequency, F/fc. For Q up to 0.90 the correlation is highly significant during control period (Fig 2*a*), blood removal (Fig. 2*b*) and blood replacement (Fig. 2*c*). Furthermore, the slopes of the three regression lines, which provide a measure of the average intraburst frequency, did not differ significantly in the experimental situations. Thus, f did not change appreciably until Q approached unity, that is, when the cell tended to fire continuously.

These changes within individual cells may explain the observations of Arnauld *et al.*[5] in the SO nucleus of the dehydrated monkey: as plasma osmotic pressure increased the predominant types of cells were first slow-firing cells, then phasic cells and finally fast, continuously-firing cells. We may conclude that the phasic pattern is a functional state of activity of a cell which at other times can be silent or firing continually. The significance of this phasic state is possibly that phasic cells are synchronised to produce an episodic discharge of neurohypophysial hormone. In normal conditions there is no obvious synchronisation of bursts[6] but overlapping activity within a population of neurones inevitably increases as the duration of bursts increases. The modulation of burst duration, which in our experiments preceded changes in intraburst frequency could provide a precise mechanism for regulating hormone release. An increase of intraburst frequency would further augment hormone release[7].

These observations do not prove that phasic cells secrete vasopressin, nevertheless we now know that they are activated by hyperosmotic injections[2], dehydration[5], carotid occlusion[8] and haemorrhage, all of which cause vasopressin release, and that they do not seem to be involved in oxytocin release[1,3]. We propose, therefore, that, on the basis of their neurophysiological performance, PV and SO neurones projecting to the neurohypophysis may be separated into oxytocinergic cells—characterised by their high frequency synchronous discharge during suckling[1,3]—and vasopressinergic cells—characterised by a tendency to fire asynchronously in phases, the duration and frequency of which determine the rate of vasopressin release.

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Induction of photoreceptor voltage noise in the dark in *Drosophila* mutant

RANDOMLY occurring, discrete voltage fluctuations (quantum bumps) have been recorded in photoreceptors of several arthropods[1-4]. It is thought that these bumps sum to produce the receptor potential[5,6]. We have investigated the relationship between photoexcitation of the visual pigment and the production of quantum bumps using a mutant of *Drosophila melanogaster*—a third chromosome recessive mutant described by Cosens and Manning[7], in which the electroretinogram decays to baseline during a strong, prolonged stimulus. We will call this mutant the transient receptor potential mutant (*trp*).

Experiments consisted of intracellular recordings from the photoreceptors of intact preparations of white-eyed *trp* mutant (*w*; *trp*). Figure 1*a* shows intracellularly recorded responses of a single photoreceptor to various intensities of green light ($\lambda = 540$ nm). Figure 1*b* shows a plot of the intensity–response relationship for the initial transient, and the subsequent steady-state phase of the responses.

In normal flies the steady state persists for the entire duration of the stimulus. The mutant response to dim light is similar to that of the wild type (Fig. 1*a*, bottom). With increased stimulus intensities, however, the mutant response has a large initial amplitude (as in the wild type), but it decays during stimulus to the same low, steady-state level observed with dim stimuli. The rate of the decay and the final steady-state level attained, vary among individual flies—the response often decaying to the dark-resting potential level.

In the *trp* mutant, voltage noise can be seen superimposed on the response at all the stimulus intensities tested (Fig. 1*a*). The noise level during the steady-state phase of the mutant response is greater than the dark noise level, and it shows little change in amplitude with increases in stimulus intensity. In contrast, in the wild type, the voltage noise amplitude greatly decreases with increased stimulus intensity. The fact that the voltage noise of relatively large amplitude is detected in the mutant response at all intensities rendered the *trp* mutant particularly useful for the present investigation.

The two upper traces of Fig. 2 are intracellular responses to weak, and to 100 times more intense light. The two lower traces are magnifications (×3) of the portions of the two upper traces indicated by vertical bars. The figure shows that the response to the weak light consists of slow potential-fluctuations (noise). Similar noise was recorded and analysed in wild-type *Drosophila*,[6] where the noise seemed to come from quantum bumps occurring at a low rate. The similarity of the noise recorded here, suggests that the mutant noise also arises from quantum bumps. The mutant response to stronger light (first and third traces), after decaying nearly to the steady-state level (Fig. 2), also displays voltage fluctuations, similar to the bump noise seen in the responses to weak light (second and fourth traces). Since bumps are expected to have their maximum amplitude when elicited from a dark-adapted photoreceptor using dim stimuli, the above result suggests that the bumps elicited in the mutants by strong light also have the same maximum amplitude after the receptor potential has decayed to nearly the steady-state level. It follows, therefore, that the decay of the mutant response during stimulus arises not from the decrease in the bump size, but from a decrease in the number of bumps composing the response.

Figure 1 shows that the steady-state amplitude saturates at a relatively low stimulus level. The saturation of the steady-state amplitude is accompanied by saturation of the response noise characteristics (Fig. 3*a*). This was demonstrated by calculating the autocovariance $C(\tau)$ (Fig. 3*a*) from the noise superimposed on the steady-state phases of the responses shown in Fig. 1, using the formula:

$$C(\tau) = \overline{[v(t) - \overline{v(t)}]\,[v(t+\tau) - \overline{v(t)}]} \qquad (1)$$

where v is the amplitude of the receptor potential in the steady-state phase, t is the time at which v was measured, and τ is the time lag. The first point, ($\tau = 0$) in the curves is the variance, a measure of noise amplitude. Once the stimulus is strong enough to be above the saturation intensity for the steady-state phase, the autocovariance shows little dependence on stimulus intensity (Fig. 3*a*). The autocovariance, calculated from the steady-state response of the same cell at even higher intensities than those shown in Fig. 3*a*, also showed little dependence on light intensity. Since the steady-state responses to stimuli of very different intensities have nearly the same amplitude (Fig. 1, log $I = -2.5$ and -1.5) and noise characteristics, that is, nearly the same variance and autocovariance (Fig. 3*a*), shot-noise analysis[5] suggests that these responses are produced by superposition of nearly the same number of quantum bumps having similar average bump amplitudes.

In *Drosophila*[8,9], as well as in other invertebrates[10–14], an intense coloured stimulus that shifts a substantial amount of the visual pigment (rhodopsin) to its thermally stable state (metarhodopsin), induces an excitatory process manifested by a prolonged depolarising afterpotential (PDA), which far outlasts the stimulus. Photoconverting the pigment back from metarhodopsin to rhodopsin induces an inhibitory process, and depresses or prevents the PDA from occurring. Induction and depression of the PDA in the *trp* mutant are shown in Fig. 3*b*. The blue light, which shifts rhodopsin to metarhodopsin, depolarises the cell, and the depolarisation is accompanied by an increase in the noise level of the response. When the blue light is switched off, the cell remains depolarised (PDA), and the noise level remains increased during the entire PDA period. The red stimulus shifts the pigment back from metarhodopsin to rhodopsin, depresses the PDA (when the light is switched off), and reduces the noise level to the level before PDA induction. Figure 3*a* shows the autocovariance calculated from the noise recorded in the dark, during PDA, from the same cell analysed in Fig. 1 and 3*a*. The similarity between the curves calculated from the light-coincident noise and the PDA noise, suggests that the PDA noise also arises from bumps.

The decay of the receptor potential is possibly caused by light adaptation. Light adaptation has been found to reduce the amplitude of bumps[5]. Figure 2 shows, however, that the bump amplitude does not decrease, while the mutant receptor potential amplitude does decrease, during illumination with stronger stimuli. Furthermore, the application of the shot-noise analysis suggests that, at the saturated steady-state level, the responses to both weaker and stronger stimuli consist of bumps of approximately the same number and amplitude. Both these

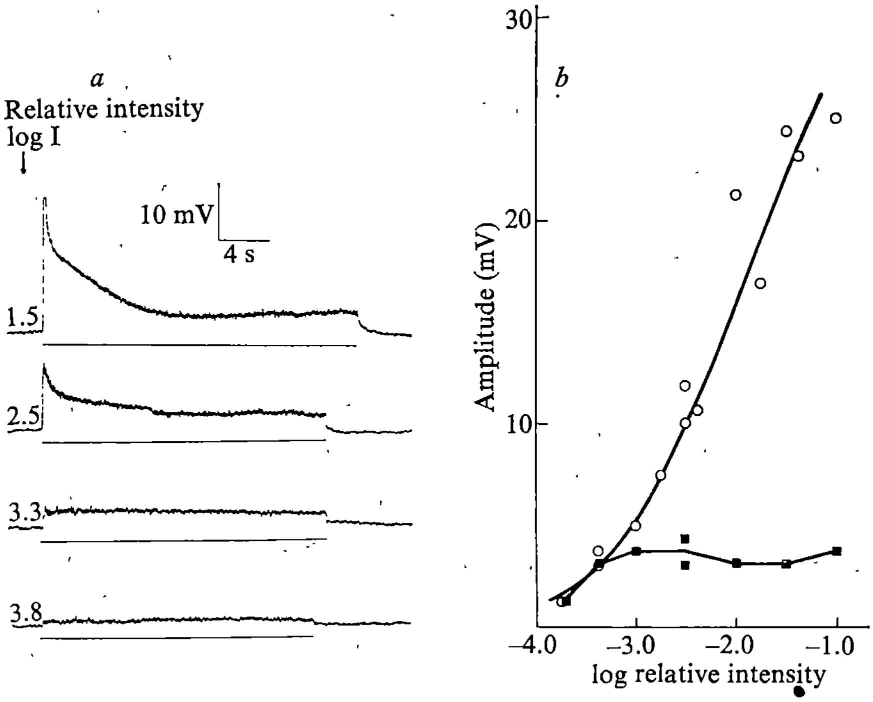

Fig. 1 *a*, Intracellularly recorded responses from photoreceptors of the white-eyed *trp* mutant to various intensities of monochromatic 540-nm green light (half peak bandwidth of 16 nm). The light source was a Xenon arc lamp used in conjunction with Bausch and Lomb high-intensity monochromator. The unattenuated illuminance was 3.0 $\times 10^{14}$ photons cm^{-2} s^{-1} at 480 nm, 3.3$\times 10^{14}$ photons cm^{-2} s^{-1} at 520 nm, and 3.6$\times 10^{14}$ photons cm^{-2} s^{-1} at 540 nm. The lines below the responses indicate the duration of the light stimulus. *b*, A plot of the amplitude–log intensity curves for the transient phase (○) and the steady-state phase (■) of the response. All data points were measured from the cell whose responses are presented in Fig. 1*a*.

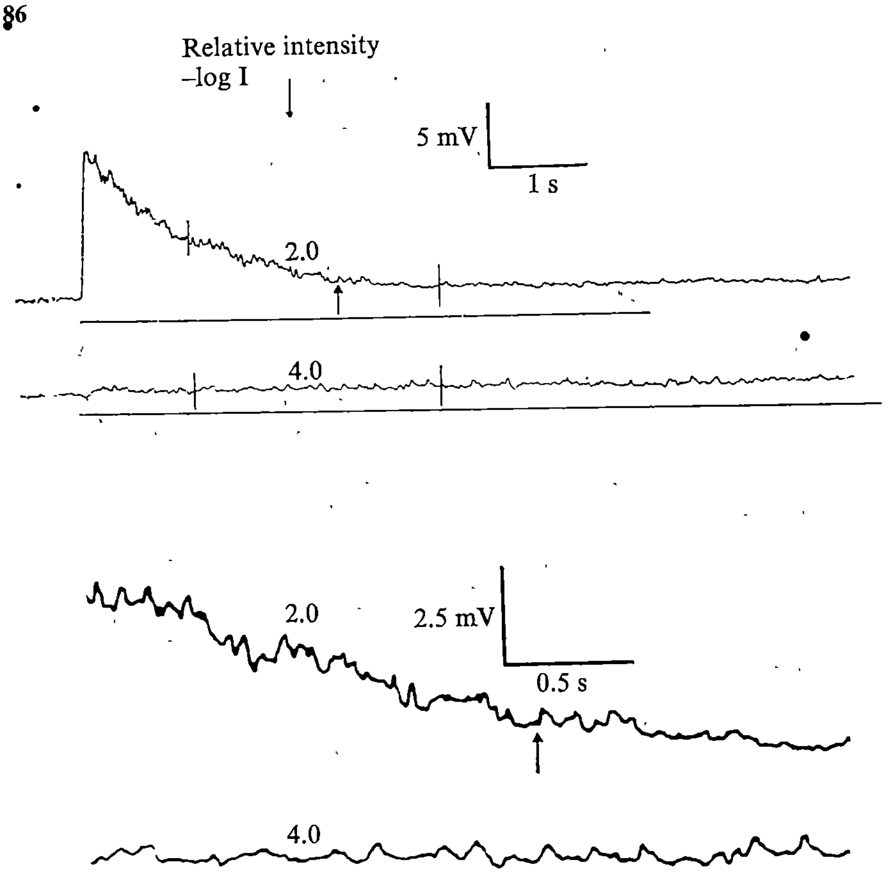

Fig. 2 Intracellularly recorded responses from a single photoreceptor of *trp* to the monochromatic 520-nm green light attenuated by 2.0 (first trace) and 4.0 (second trace) neutral density filters. The two lower traces are ×3 magnifications of the marked portions of the two upper traces. The responses were filtered with a high frequency cutoff filter (half power cutoff at 40 Hz), to facilitate observation of bumps. The arrows indicate the approximate time after which, the response to the stronger light shows noise characteristics similar to the response to the weaker light.

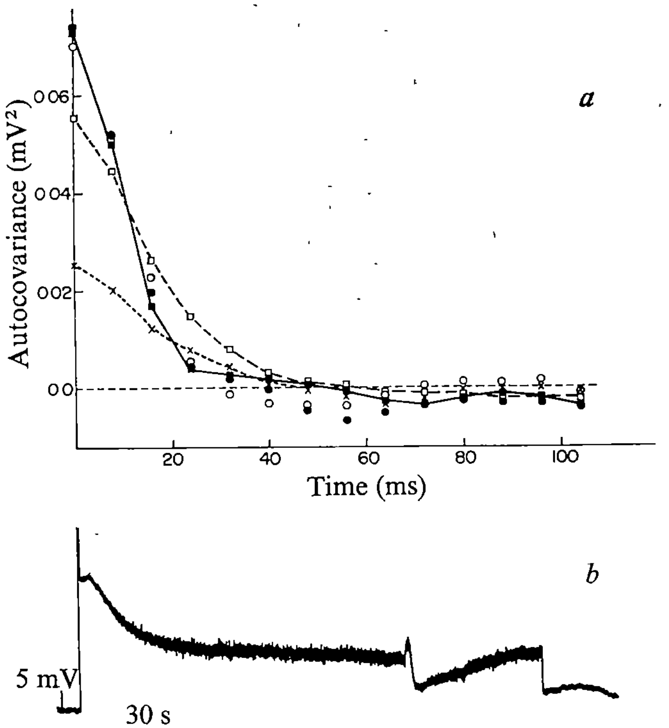

Fig 3 *a*, Autocovariance functions calculated from dark noise (×), the steady-state responses (□, −log I = 3.8; ■, −log I = 2.5; ○, −log I = 1.5) shown in Fig. 1 and the PDA recorded from the same cell (●). The calculations were done from 750 points sampled at 8-ms intervals The ordinate is the autocovariance $C(\tau)$ in mV² and the abscissa is the time lag τ in ms. The responses were filtered with a high frequency cutoff filter (half power cutoff at 30 Hz) before the analysis. Calculation without filtering gave similar results. *b*, An intracellularly recorded PDA response from the photoreceptor of the mutant *trp*, induced by blue and depressed by red stimuli The stimuli originated from a quartz iodide lamp filtered through Baird Atomic B3 interference filters, having transmission peaks at 480 nm and 600 nm. Illuminances were 4.0×10^{16} photons cm^{-2} s^{-1} and 2.5×10^{16} photons cm^{-2} s^{-1} for the blue and red stimuli, respectively.

results make the light adaptation hypothesis improbable. Another possible explanation is that the decay of the receptor potential occurs in the mutant *trp*, because the stimulus bleaches a substantial fraction of visual pigment, and the pigment does not regenerate normally. This explanation, too, is unlikely because the decay of the potential to the steady-state level occurs at intensities that activate less than 1–3% of the visual pigment, when measured spectrophotometrically or by using a response similar to the early receptor potential[15]. We therefore suggest, on the basis of the results shown in Fig. 2, that the decay of the receptor potential during a stimulus arises from a reduction in the quantum efficiency of bump production. The fact that individual bumps in the mutant appear to be normal, although their number is reduced, suggests that the mutation affects an intermediate step of phototransduction, and the fact that PDA induction in the *trp* mutant is accompanied by the induction of bump noise after the cessation of stimulus, suggests that the coupling between the photoexcitation of the visual pigment and the production of the bump is not direct, and that the PDA and the stimulus-coincident response share some common pathway.

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Possible contribution of ionic clustering to molecular packing of collagen

An analysis of the primary structure of the α1 chain of a collagen demonstrated that electrostatic and hydrophobic interactions between collagen molecules were maximal when the rod-like molecules were staggered by 234*n* amino acid residues, where *n* is 0, 1, 2, 3 or 4 (ref. 1). It was not possible, however, to determine what value of *n* was most likely. This information would help to select from among possible molecular packing models[2,3]. Doyle *et al.*[4] re-examined the electrostatic interactions in an attempt to determine *n*. Since amino acid side chains of opposite charge frequently occur close to each other in the sequence[1], they considered that these potential intrachain ion pairs might function as dipoles with approximately the same direction in the molecule as in the sequence. If antiparallel dipoles on adjacent molecules were attractive (Fig. 1*a*) and parallel dipoles were repulsive, they found that the net number of attractions was maximal at $n = 1$ and negative at other values of *n*. They concluded that dipole–dipole interactions were important in stabilising the native fibril and that the Smith microfibril[5] was favoured over other packing models since it is essentially a helix of collagen molecules staggered by 234 residues between nearest neighbours.

Although we find the assumptions made by Doyle *et al.*[4] difficult to accept, they were apparently able to discriminate from among possible values of *n*. This suggests that the reason should be more carefully examined. We discuss here other aspects that may be important and propose as an alternative concept that ionic clustering may contribute significantly to the stabilisation of collagen fibril structure.

First, interactions between dipoles cannot be estimated when the location of the charges is not well defined. This point is of particular importance in the case of collagen. The charges are at the ends of side chains, which may assume many configurations, and there are generally three sets of charges at any level since there are three similar or identical chains in the molecule. Therefore, neighbouring charges of opposite sign in a single chain will not necessarily form a dipole in an actual structure, and even if dipoles are formed, the contributing ions and their orientation would be difficult to predict. A related conclusion is that the ions would be able to arrange themselves so as to minimise electrostatic repulsion, which then would not be a major factor, contrary to the assumption of Doyle *et al.*[4].

Second, there is information in the sequence, not explicitly used by Doyle *et al.*[4], that explains their result without recourse to a consideration of dipole–dipole interactions. Not only are amino acid side chains of opposite charge found close to each other in the sequence much more often than would occur by chance, but glutamic acid and arginine are not equally distributed between position X and Y in the collagen triplet Gly–X–Y, with the result that about three out of four positive charges precede glycine and about three out of four negative charges follow glycine in the sequence. Therefore triplets of the form Y–Gly–X and Gly–X–Y, where Y has a positively charged side chain and X has a negatively charged side chain, are common. Of the fifty 'dipoles' considered by Doyle *et al.*[4], fifteen are of the former type and thirteen are of the latter type. This geometry suggests an arrangement (Fig. 1*b*) of four alternating unit charges approximately in a line that could result in cooperative electrostatic interactions with a lower potential energy than two separated ion pairs and a more favourable stereochemical relationship than the two 'dipoles' in Fig. 1*a*. An examination of molecular models shows that such an arrangement is possible because of the separation of the one pair by a glycine residue. Furthermore, the arrangement provides a reason for the unusual distribution of glutamic acid and arginine. Similar arrangements of other opposing antiparallel charge pairs can be devised. This approach to the location of ionic groups leads to the conclusion reached by Doyle *et al.*[4] that $n = 1$, if the same scoring system is used. If, however, the possibility of repulsive interactions is de-emphasised and other arrangements of charges are allowed, as we argue, a most probable value of *n* can no longer be selected with confidence by this analysis.

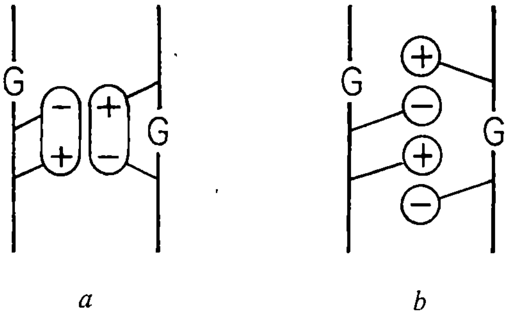

Fig. 1 *a*, Antiparallel dipoles between collagen molecules considered to be attractive by Doyle *et al.*[4]. *b*, An alternative arrangement of the ions to form a line of alternating charges. G is glycine. The positive and negative ions show two characteristic orders of charged amino acids in the triplet sequence of collagen chains.

The considerations discussed here suggest a concept that may help in understanding how collagen fibrils are stabilised. The argument is as follows. Since the collagen molecule has three similar or identical chains as already noted, and since more than one chain per molecule may contribute groups to interacting edges between molecules, an arrangement of the type shown in Fig. 1*b*, if viewed in three dimensions, could involve additional ionic groups. Furthermore, there are often more than two ionic groups near each other in the sequence, and it is possible that an interesting region may involve more than two molecules. Therefore, clusters of ions may exist. Schematic representations of the Smith microfibril suggest that as many as 16–20 ions could cluster in ordered arrangement. These would be expected to approximate the properties of an ionic crystal and contribute some measure of stability to the collagen fibril structure analagous to crystal field energy. This could be an especially important contribution if the clusters were in the interior of the Smith microfibril, or one of the similar alternative structures, since the movement of water, other small molecules, and amino acid side chains would be restricted, increasing the likelihood of cooperative ordering and depolarisation. A more complete analysis may settle some of the details of the molecular packing of collagen, such as the value of *n*, but this has not yet been done.

The principle of ordered ionic clustering proposed here may apply generally to supramolecular structures, which create extended regions where movement is restricted. Guss *et al.*[6] have found an example by X-ray diffraction of a helical form of hyaluronic acid which has a line of alternating charges of opposite sign down the centre of the helix.

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Mobile charge carriers in photosynthesis

CURRENT mechanistic models of energy transduction in photosynthesis, envision that the energy of the photons collected by the bulk antenna chlorophyll, is funnelled to special sites called reaction centres, where the primary charge separation events occur[1]. If the primary oxidant and reductant are separated physically by only a few, or few tens of angstroms, changes in the dielectric properties should become evident[2]. Several laboratories have sought such evidence with equivocal results[3-6], although one group, using methods similar to ours, has reported qualitative, positive results[7].

We report here direct confirmation of the presence and trapping of charge carriers of both signs, their mobilities and relative quantum yields, and an estimate of the distance between the separated units of charge.

Our experiments measured the light-induced changes in the real and imaginary parts of the complex dielectric constant, $\varepsilon = \varepsilon' + i\varepsilon''$, of photosynthetic samples at the microwave frequency 9 GHz. Changes in the imaginary part of the dielectric constant, ε'', reflect the losses or photoconductivity, $\Delta\sigma$, whereas changes in the real part, ε', can be related to light-induced changes in the polarisability, α, from which a distance can be estimated[8-10]. The signs and Hall mobilities of the carriers were determined by measurements of the light-induced Faraday rotation. The apparatus consisted of a phase coherent microwave instrument including a bimodal cylindrical transmission cavity[11], the design and performance of an earlier version have been reported previously[12].

The biological samples consisted of films of whole and broken chloroplasts from plants and algae, and chromatophores from photosynthetic bacteria. The samples were prepared according to well established experimental procedures[13,14], and the films were obtained by evaporation, in a mild air flow, of aqueous suspensions of the photosynthesis systems. Partial dehydration of the samples did not alter the primary photo-events, which were monitored by the measurement of light-induced electron paramagnetic resonance signals[15,16], and of light-induced absorbance changes, indicating bleaching of the reaction centre pigments[1,16]. The films were deposited on microscope cover slips, and were located, in the microwave cavity, at the antinode of the microwave electric field, by means of styrofoam supports. Light pulses obtained from a Xenon flash tube or a ruby laser were fed into the cavity by means of quartz light pipes. Absolute light intensities were measured at the sample location by fast silicon light detectors, with calibrations traceable to the USA National Bureau of Standards.

Our primary observables were the light-induced changes in the microwave power coupled through the bimodal transmission cavity, which are related to the photoconductivity[8]. Accompanying these, there may be changes in the real part of the dielectric constant, which are reflected as changes in the resonance frequency of the cavity[9]. On application of an external magnetic field, with particular adjustments of the bimodal cavity, the power coupled through the cavity is related to the Faraday rotation of the sample[11]. When the cavity is imperfectly adjusted the photoconductivity and Faraday rotation signals are superimposed, but can be separated.

Figure 1*a* is an example of a photoconductivity signal, $\Delta\sigma$, from a sample of chloroplasts of *Spinacia oleracea*. As $\Delta\sigma = \Delta n q \mu$, where Δn is the change in number of carriers of charge, q, and mobility, μ, it is necessary to determine μ to obtain values of Δn; otherwise the data are only qualitative[7]. Light-induced changes in carrier mobility were negligible, and thus did not contribute significantly to the observed photoconductivity. Estimation of the quantum yield for carrier generation, $QY = \Delta n$/quanta absorbed, required knowledge of the changes in carrier concentration Δn, and the quanta absorbed by the sample. The Hall mobility, $\mu_H = \theta_H/B$, can be obtained from measurements of the Faraday rotation, where θ_H is the Hall angle, or angular rotation of the carrier current on application of an external magnetic field of intensity B. Figures 1*b* and 1*c* are examples of Faraday rotation signals, for a value of $B = 10^4$ gauss, superposed on photoconductivity signals. In Fig. 1*b*, the settings were such that a negative carrier would produce an upward deflection, positive carriers would produce a downward deflection. These Faraday rotation signals add algebraically to the photoconductivity signal of Fig. 1*a*. The early temporal component in Fig. 1*b* is larger in magnitude than the corresponding portion of Fig. 1*a*, thus establishing its origins in negative carriers. The later temporal components in Fig. 1*b* are of opposite sign to the early component, and subtract from the photoconductivity signal of Fig. 1*a*. They are thus attributed to positive carriers. In Fig. 1*c*, the experiment was repeated with the direction of the external magnetic field reversed. The field-dependent components reverse their deflection directions, and the early temporal components subtracts from the photoconductivity signal of Fig. 1*a*, whereas the latter components add to it. The sum of Fig. 1*b* and 1*c* removes the magnetic field-dependent signals, while their difference removes the photoconductivity signal. The reversal of the field-dependent signals on reversal of the sign of the external magnetic field, established the origins of these signals as Faraday rotations.

The Hall mobilities as calculated from Fig.1 are of the order of 1 $\text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$. Using this value of μ, we calculated the number of positive carriers as $10^{13}\ \text{cm}^{-3}$ for saturating intensities of actinic light, and quantum yields around 1% for non-saturating illumination levels.

Data, similar to those shown in Fig. 1, have been obtained from leaves and chloroplasts of other green plants and algae, and from chromatophore fragments of the photosynthetic bacterium *Rhodopseudomonas spheroides*. Notably, the non-photosynthetic mutant of this organism, PM-8, gave rise to signals one to two orders smaller in magnitude, than those exhibited by the photosynthetically active varieties, and did not exhibit the sign reversal in the Faraday rotation experiments.

Figure 2 shows the wavelength dependence of the microwave photoconductivity signals for a sample of spinach chloroplasts similar to that from which the data of Fig. 1 were obtained. This dependence generally parallels that for photosynthesis[18], aside from an increase towards the longer wavelength, where the reaction centre pigments absorb. A similar long wavelength

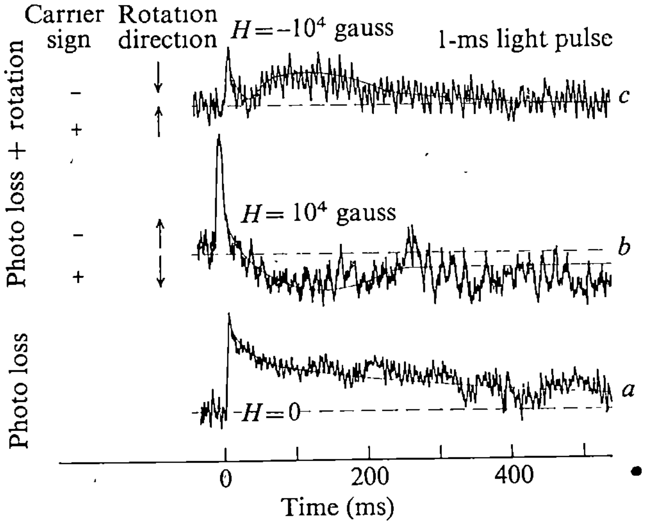

Fig. 1. Microwave photoconductivity and Faraday rotation signals from a spinach chloroplast film The photoconductivity signal, trace *a*, exhibits both fast and slow components. In *b* a Faraday rotation signal adds algebraically to the photoconductivity of *a* so that the early temporal components attributable to negative carriers add while the later components subtract. For the opposite direction of magnetic field, *c*, the Faraday rotation signals reverse sign while the photoconductivity maintains the original sign, thus leading to a subtraction of the signal for the negative carriers.

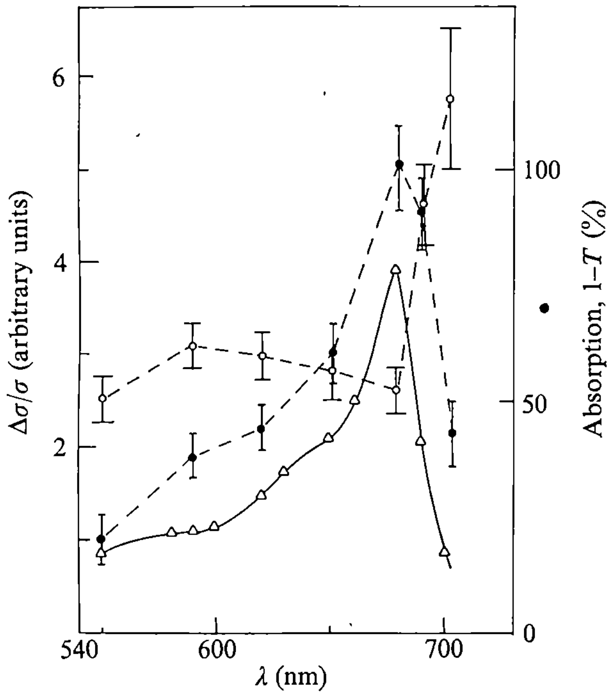

Fig. 2. Action spectrum and relative quantum yield for the microwave photoconductivity in a spinach chloroplast film. △, Absorption spectrum of the sample; ●, action spectrum, ○, relative quantum yield. The points are normalised to constant incident light intensity at 550 nm.

increase was also observed in the photosynthetic bacteria.

Superposition of pulsed light on steady background illumination, is a standard procedure in semiconductor physics to study trapping processes[10]. Such procedures were used in our experiments; they provided evidence for the trapping of carriers of both signs, and facilitated a rough determination of the trap concentration of ~ 10^{13} cm^{-3}. The number of reaction centres, estimated from the chlorophyll content of the sample, was also ~ 10^{13} cm^{-3}. Arrhennius plots of the temperature dependency of the photoconductivity signals and their decay times, gave activation enthalpies of 0.3 eV.

Light-induced changes in the real part of the dielectric constant, $\Delta\varepsilon'$, of ~ 10^{-5}, were observed. Changes in the dielectric constant can be related to changes in volume polarisability, α, using the Clausius–Mossotti relationship[10]. From this value of $\Delta\varepsilon'$, we calculated a value of $\Delta\alpha$ of 10^{-20} cm^3. Assuming that α approximates the volume over which the charges are separated, $\alpha^{1/3} = 3\times10^{-7}$ cm, represents a separation length. This value is comparable with the presumed size of the pigment–protein complex of the reaction centre[19,20].

In the bacterial chromatophores the light-induced ESR signals exhibit decay half-life regimes of 30–50 ms, and of several seconds; the positive carriers exhibit very similar kinetic components. The reaction centreless mutant, *PM-8*, exhibits neither Faraday rotation, nor photo-induced ESR signals. Titration of the chromatophores with ferricyanide to maximum dark ESR signal intensity, concurrently eliminates the slow components of the photoconductivity signals. Taken together, these observations point to a common origin for the ESR and positive carrier photoconductivity signals.

The positive carrier component is thus interpreted as originating in the same species that produced the ESR signal I, the dimeric chlorophyll cation radical[20,21], and we suggest that this species behaves as a mobile hole (over a volume of 30° $Å^3$), when observed at microwave frequencies. The negative carrier component of the photoconductivity (few ms range) is tentatively interpreted as arising from electrons thermally released from the primary acceptors (the reverse process), and its decay as resulting from charge transfer to secondry acceptors; because of their larger energy gap, they would not contribute significantly to the population of the conducting state. Rigorous interpretation of the observed activation enthalpies is seriously hindered by the fact that they may represent a combination of activations for both carrier types, and it is not possible to separate their contributions from the available data.

The general behaviour, therefore, can be understood in terms of photoliberated charges migrating for short distances through a dielectric medium. The microwave Hall mobilities are incompatible with charge transport through conduction bands; rather, they are similar to those expected for tunnelling or hopping processes[10].

These observations and interpretations, which will be elaborated fully elsewhere, strongly support the model in which the primary charge separating events occur in the reaction centres in photosynthesis. The fact that such signals are not observed in the reaction-centreless mutant of *R. spheroides*, together with stringent controls, and other evidence not reported here, rules out an artefactual source of the signals. The action spectra for the microwave photoconductivity, resembling, as they do, those for photosynthesis, point to a close connection between the two processes. The number of traps, as well as the maximum number of free carriers, approximates the number of reaction centres, thus connecting the observations with the structure of the photosynthetic unit.

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Kinetic evidence for a conformational transition in rhodopsin

AFTER light absorption, rhodopsin decays through a series of spontaneous reactions[1–10], the kinetics of which have been the object of considerable study. In a variety of conditions the decay kinetics of bathorhodopsin (prelumirhodopsin), lumirhodopsin, and metarhodopsin I (meta I) have been found to be non-first order, but are consistent with a set of parallel, independent first-order reactions[2–10]. These kinetics have been assumed to result from the existence of multiple forms of each of these intermediates, and it has been proposed that rhodopsin itself exists in different forms or different environments in some conditions[7]. The kinetics of meta I → meta II in both dodecyltrimethylammonium bromide- and digitonin-solubilised phospholipid-free rhodopsin are also consistent with two forms of meta I which decay independently (J. G. S. and E. O. Plante, unpublished). We show here that the effect of temperature changes on these kinetics is consistent with the hypothesis that rhodopsin exists in two forms in equilibrium resulting from the accessibility of two stable conformational states separated by a small difference in free energy.

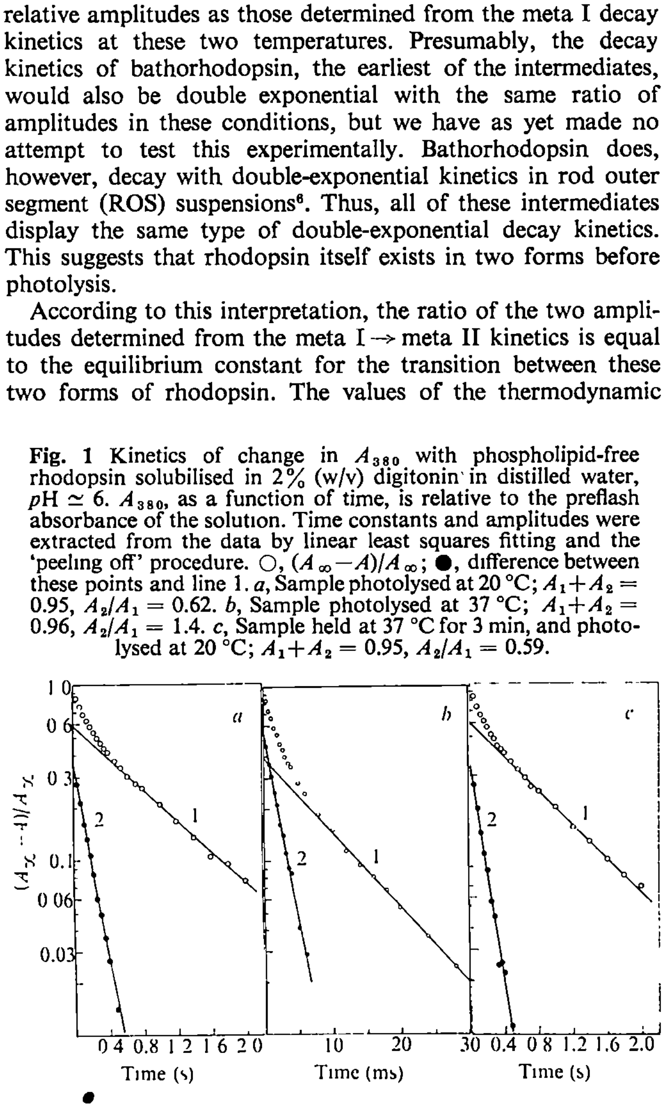
We have studied the kinetics of rhodopsin decay reactions using flash photolysis of detergent-solubilised rhodopsin containing less than 0.2 mol phospholipid per mol rhodopsin. The kinetics of meta I → meta II were determined from absorbance measurements at 380 nm and of lumirhodopsin → meta I from absorbance measurements at about 415 nm, which is near the isosbestic point of meta I and meta II. In each case the absorbance against time function could be fitted by a sum of two exponential decay functions of the form $\Sigma A_i \exp(-t/\tau_i)$. We used a phospholid-free preparation so that we could exclude phospholipid heterogeneity of the digitonin micelle as a source of multiexponential kinetics.

We have assumed that these kinetics result from the independent decay of two forms of each of these species, and thus that the amplitudes of the two exponential terms are proportional to the initial concentrations of these two forms. Figure 1 shows that for meta I → meta II a change in temperature from 20 to 37 °C results in a change in the ratio of these two amplitudes, but not in their sum (Fig. 1*a* and *b*). Furthermore, this change is reversible (Fig. 1*c*). Such kinetics are caused by two components which are interconvertible, but only on a time scale which is long compared with the time constants for decay of meta I. Thus, these two components exist in equilibrium, but this equilibrium is established before the appearance of meta I.

We also found that the kinetics of decay of the earlier intermediate, lumirhodopsin, at 20 and 30 °C were consistent with double-exponential kinetics with approximately the same relative amplitudes as those determined from the meta I decay kinetics at these two temperatures. Presumably, the decay kinetics of bathorhodopsin, the earliest of the intermediates, would also be double exponential with the same ratio of amplitudes in these conditions, but we have as yet made no attempt to test this experimentally. Bathorhodopsin does, however, decay with double-exponential kinetics in rod outer segment (ROS) suspensions[6]. Thus, all of these intermediates display the same type of double-exponential decay kinetics. This suggests that rhodopsin itself exists in two forms before photolysis.

According to this interpretation, the ratio of the two amplitudes determined from the meta I → meta II kinetics is equal to the equilibrium constant for the transition between these two forms of rhodopsin. The values of the thermodynamic parameters for this hypothetical transition were calculated from the temperature dependence of the equilibrium constant in the range 20–37 °C (Fig. 2). For phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, $pH \simeq 6$, $\Delta H^0 = 9.8$ kcalorie, $\Delta S^0 = 32$ e.u., and at 37 °C, $\Delta G^0 = -0.2$ kcalorie. These values are of approximately the same magnitude as those reported for transitions between some conformational substates of α-chymotrypsin[11,12]. Two components in these kinetics could also be due to the availability of two different environments in the digitonin micelle, but we have recently obtained similar results with ROS solubilised with cetyltrimethylammonium bromide, Emulphogene BC720, Amonyx-LO and with detergent-free suspensions of sonicated ROS. Thus, it seems likely that the heterogeneity inferred from these kinetics resides in the state of the protein itself.

Fig. 1 Kinetics of change in A_{380} with phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, $pH \simeq 6$. A_{380}, as a function of time, is relative to the preflash absorbance of the solution. Time constants and amplitudes were extracted from the data by linear least squares fitting and the 'peeling off' procedure. ○, $(A_\infty - A)/A_\infty$; ●, difference between these points and line 1. *a*, Sample photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.62$. *b*, Sample photolysed at 37 °C; $A_1 + A_2 = 0.96$, $A_2/A_1 = 1.4$. *c*, Sample held at 37 °C for 3 min, and photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.59$.



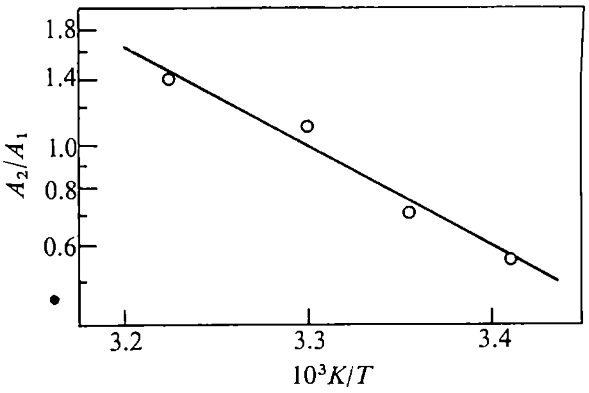


Fig. 2 Van't Hoff plot for the proposed reaction $R_1 \rightleftharpoons R_2$ over the range 20–37 °C. At each temperature, A_1 and A_2 were determined as described in Fig. 1. A straight line was fitted to the data points by linear least squares, giving $\Delta H^0 = 9.8$ kcalorie and $\Delta S^0 = 32$ e.u.

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Erratum

In the article "Seismic evidence for local undulation of olivine–spinel phase boundary", by S. K. Dey-Sarkar and R. A. Wiggins (*Nature*, **257**, 572; 1975) the last two sentences of the first paragraph should read: While analysing seismograms between 14 and 18°, a group of anomalous data was observed localised in a specific region which could be explained only by a local undulation of the 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation.

obituary

John Morrison Barnes, CBE, Director of the MRC Toxicology Unit, Carshalton, died after a short illness on September 24 at the age of 62.

Dr Barnes studied medicine at the Universities of Cambridge and Sheffield and graduated in 1936 He received his early training in research in the laboratory of Howard Florey at Oxford, where he studied lymphocytes and also carried out very important initial toxicity tests on penicillin. He then collaborated with J. Trueta on work related to war injuries, including research on limb ischaemia, the absorption of bacterial toxins from tissues and the treatment of burns. He joined the Royal Army Medical Corps in 1942 and spent the next three years at the Defence Establishment at Porton, with which he remained closely associated as an adviser for the rest of his life. In 1947 he was responsible for setting up the MRC Toxicology Unit at Carshalton which, from very small beginnings, grew under his directorship to become one of the largest establishments of the MRC. The guiding principle of nearly all the research at the Toxicology Unit was derived from the concept of Claude Bernard that toxins could be used to elucidate physiological processes coupled with the ideas of the biochemical lesion and of lethal synthesis, due to Peters Many toxic substances were studied, Barnes himself being particularly interested in the toxicology of beryllium, organophosphorus compounds, organotin compounds, nitrosamines, aflatoxin and carbon disulphide. Barnes was perhaps best known internationally for his work on organophosphorus and carbamate pesticides. The Unit became a World Health Organisation International Reference Centre for the Evaluation of the Toxicity of Pesticides in 1967 and Barnes advised the Ministry of Agriculture on medical aspects of pesticides for many years. He produced an invaluable guide, *Toxic Hazards of Certain Pesticides to Man* (WHO Monograph 16). Because of his impressive depth of scientific knowledge combined with his great practical common sense, Barnes was uniquely in demand as an advisor on all aspects of possible hazards from environmental and occupational exposure to toxic chemicals. He was a member of numerous Governmental and other advisory committees including the British National Committee for Problems of the Environment. He served on the editorial boards of several scientific journals and was made Visiting Professor of Pharmacology at King's College, London in 1967. Barnes had considerable skill as a laboratory bench worker but little opportunity to exercise it in his later years because of the punishing load of advisory and administrative work that he willingly undertook. His unobtrusive help and guidance was greatly valued by his colleagues and had he been less unselfish, his output of original published work could have been much greater. John Barnes contributed more to the establishment of toxicology as a scientific discipline than anyone in this country and perhaps in the world.

P. N. Magee

Professor J. C. Pócza, Head of the Department of Structure Research of the Research Institute for Technical Physics of the Hungarian Academy of Sciences, Budapest, died on September 10. Born in 1915, he graduated from the University of Szeged in 1938 and obtained his Ph.D. at Budapest University in 1945.

Professor Pócza first worked on structure determination by X-ray diffraction and then, as assistant at the Institute of Nuclear Physics, took part in the first lunar-echo measurements, carried out under the direction of Professor Z. Bay.

He entered the field of thin film physics in 1954, studying the thin-film growth processes by electron microscopic *in situ* methods. He was a member of the International Committee on Thin Films, a member of the Editorial Board of Thin Solid Films, and was the Chairman of the 3rd International Thin Film Conference held in Budapest this summer.

For many years he was the deputy general secretary of the Hungarian Eötvös Loránd Physical Society and a member of the Hungarian National Committee of IUVSTA.

J. C. Anderson

announcements

Award

The 1975 **Premio Modesto Panetti** has been awarded to **R. S. Rivlin** for his work in the field of mechanics.

Appointments

J. L. Harper, J. S. Sawyer and **E. A. Vincent** have been appointed new members of the Natural Environment Research Council.

International meetings

November 12, **A new look at pollination,** Kent (The Principal's Secretary, Wye College, Ashford, Kent, UK).

November 12, **Life-games with glass beads and molecules,** Cambridge (The Secretary, Department of Chemistry, Lensfield Road, Cambridge, UK).

November 21, **The scientist as a leader,** London (The Industrial Society, PO Box 1BQ, Robert Hyde House, 48 Bryanston Square, London W1H 1BQ).

December 2–3, **Grass weeds,** Paris (European Weed Research Society, c/o ARC Weed Research Organisation, Begbroke Hill, Yarnton, Oxford OX5 1PF, UK)

Person to Person

London–Rio de Janeiro exchange. London house available for one year from about February 1976, in exchange for house or flat in Rio de Janeiro. Four bedrooms and garden (Dr C. J. Sanderson, 26 Olive Road, Cricklewood, London NW2 6TX, UK; (01) 452 8364)

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office The section will include exchanges of accommodation, personal announcements and scientific queries We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

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nature November 13, 1975

Asking questions about university research . . .

UNIVERSITY bashing is a popular pastime in the UK at the moment. True, all forms of public expenditure are bound to come under scrutiny when a country is trying to keep control of a serious economic situation, but the universities are rather better than average sitting targets because it is much easier to question the expenditure of several hundred million pounds on them than to justify it in a way that convinces the holders of the purse strings. On a slightly more detailed level, asking a question like: "Why do that particular kind of scientific research?" is easy; answering it is not.

Nobody pretends, of course, that one can find out much about as complex a beast as 'university research' by talking to two, or even ten, people. The Science Subcommittee of the House of Commons Select Committee on Science and Technology, for example, must be truly fed up with asking a question of a witness, only to be told that the query should be addressed to someone else (usually unspecified) because it is outside that witness's brief, remit or responsibility. Equally, 'ologists' of the Select Committee on Science and Technology often feel that key questions are not asked or pursued—often, it must be added in the select committee's defence, because time simply does not permit.

The objections of many of those who carp about the amounts spent on scientific research in universities stem from the supposed antipathy between universities and industry. The stereotyped picture is one in which industry complains that university training in research often does not suit the industrial environment; the universities reply that a major part of their function is to acquire new knowledge and that industry should not expect a model industrial scientist to step off a university production line.

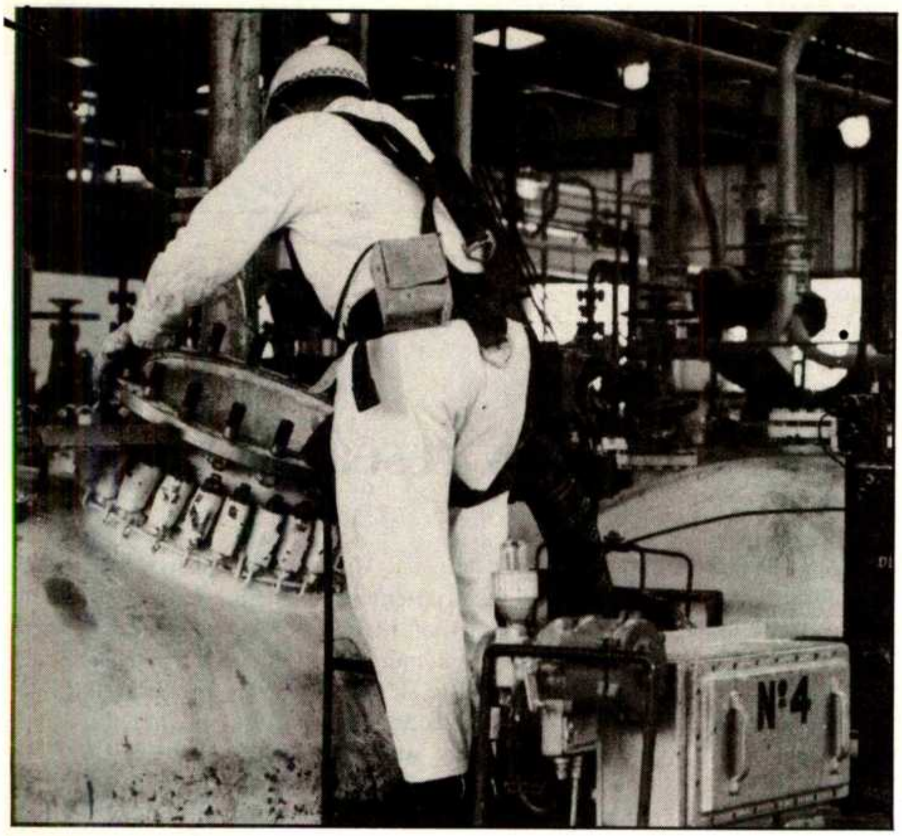
Fortunately things are not nearly as polarised as that, but there certainly are problems, and it is therefore a particularly important event when the select committee talks to industry, be it the Confederation of British Industry (CBI) or individual industrialists, about university research. It so happens that the select committee had the CBI before it last week, and one is therefore prompted to set up a list of some of the more important matters that might be covered in future select committee discussions with industry. Some of these came up at the recent meeting; some did not.

- How can the link between 'research' and 'application' be made less tenuous?
- How can the requirements of industry for, broadly, 'trained' people be squared with the universities' aim of producing 'educated' people?
- To what extent is university research compatible with the goal of much of industrial effort ('improvement' as opposed to 'innovation')?
- In what light do industrialists regard polytechnics? Should they be encouraged at the expense of (some) universities?

. . . at Cambridge in particular

IN the concluding remarks of a paper on 'useful' research carried out at the University of Cambridge (distributed from the University Offices) the late Professor J. W. Linnett said: "I have been surprised at the amount and range of useful research that is being done at Cambridge over a very wide spread of disciplines", when 'usefulness' is judged "in economic and social terms of a simple and direct kind".

This is an important statement for a man who was until a month or so ago Vice-Chancellor of the University of Cambridge to have made, even allowing that he had set out to tackle the problem of bolstering up the public image of universities. Interestingly, the standard university response to critics is also contained in Professor Linnett's paper: "Universities are places where the spirit of unfettered imaginative enquiry should be fostered". Adding his two statements together leads to a very important conclusion—that unfettered imaginative enquiry in a university environment can be of considerable use in a tangible way.



Preparing polymerisers for cleaning at BP factory.

Cutting out cancers

THE UK Government recently accepted a Convention and a Recommendation, drawn up at last year's International Labour Conference, on 'international standards concerning the protection of workers against carcinogenic substances or agents'. Both documents will play their part in establishing some useful principles and eliminating past abuses. But the practical achievement will fall short of what is usually understood by 'international standards', and the proportion of actual cancers prevented is likely to be only a fraction of the potential number. Laura Swaffield reports.

THE terms set out by the International Labour Organisation (ILO) must be seen in context. First, the documents can apply only to substances known to be carcinogenic. Unfortunately, the probability is that most industrial carcinogens have not been identified as such.

The International Agency for Research on Cancer takes it as a rule of thumb that 80% of all cancers are environmentally induced, the most easily traced often by chemicals at work. According to the WHO, however, adequately documented cases of occupational cancer represent only a fraction of 1% of the total.

The medical profession's notorious preoccupation with finding a dramatic breakthrough cure is partly to blame. A sub-committee of the US National Cancer Advisory Board, which expressed its astonishment in March of this year that "the National Cancer Program does not appear to have accorded an adequate priority nor sense of urgency to the field of environmental carcinogenesis", estimated research expenditure on preventive work as "perhaps 10% of the budget"—a pattern which is fairly typical worldwide, and not just in cancer research.

Second, the ILO documents allow great latitude to the nations concerned—each, for instance, decides for itself which carcinogens are to be prohibited or controlled, and the nature of the controls.

Third, sheer economic reality places limits on how much can be done in industry. This is one reason why individual nations are given so much freedom to decide priorities.

The whole matter came under discussion at this year's XVIIIth International Congress on Occupational Health, held in Brighton. Dr E. Mastromatteo of the ILO said the future need was for international agreements on exposure levels to chemicals. But Mr M. El Batawi from WHO was more aware of financial, political and economic problems, especially in underdeveloped countries. International exposure levels, he said, were neither practicable nor desirable—but standardised international methodology could perhaps establish a 'dose-response curve' along which each government could set its own limits.

Two case histories from the UK illustrate the bounds between which a line must be drawn. In 1927, ICI patented Nonox S, an anti-oxidant containing 2-naphthylamene which was marketed to the rubber industry. By 1938, the Association of British Chemical Manufacturers had accepted that there was a cancer risk from 2-naphthylamene. ICI started screening its own Nonox S workers, and by 1942 had established a proved cancer hazard for those working with the hot finished product. Only in 1949 was Nonox S withdrawn from sale.

The story does not end there. There was no warning to customers, and one, Dunlop, waited until the mid-1950s to set up a research unit, and until 1960 to recommend the screening of employees who had worked with Nonox S. At one plant, screening did not begin until 1965. By 1970, some 450 cases of bladder cancer had come to light many too late for effective treatment.

As late as this year, the National Health Service (NHS) distributed a circular setting out the screening arrangements for workers and ex-workers in the rubber industries. Some workers who left their jobs before screening started must still be unaware they are at risk. Meanwhile, oddly enough, the NHS does not want the circular to attract great publicity because it has "limited application", even though its implications are vast and of great discredit to all concerned.

The second case, the big occupational cancer story of 1974–75, involves vinyl chloride monomer (VCM) and shows the other side of the coin. Employees' organisations were consulted from the start when a link was discovered between VCM (used in the manufacture of PVC) and angiosarcoma, a rare liver cancer. The discovery, typically, came by accident during animal research on a VCM-linked hand condition. By early 1974, epidemiological work had turned up a possible four VCM-linked angiosarcomas in the USA, and one in the UK.

The ensuing events are something of a demonstration of ideal industrial practice. The chemical industry and government agencies (in the UK, the Employment Medical Advisory Service of the new Health and Safety Commission) got together, with trade union involvement, to find out the extent of the danger and eliminate it. The then medical adviser to the Trades Union Congress (TUC), pointed out from the start that there was no case for shutting down the polymerisation process, which annually produces 10 million tons of PVC world-wide.

By June 1974, the Department of Employment was drawing up a code of practice limiting exposure to VCM vapour to 50 parts per million with a time-weighted average of 25 ppm. As late as 1959, the prescribed threshold limit value had been 500 ppm. Significantly, the code of practice required results of atmospheric sampling to be available to workers. Today, the limit in industry is fixed at 30 ppm. (time-weighted average 10 ppm.), which is thought to be the ultimate with existing plant.

This shows what industry can do if it tries. But how does the balance sheet look? In the UK alone, £13 million has been spent since 1974 on research and alterations to plant. Four companies with a total of 500 workers are involved.

But the total of deaths in the UK now found to have been due to VCM-induced angiosarcoma is two—one a man of 71, the other a man with a long history of liver abnormality. The world-wide score is under 40, not all of them confirmed.

With thousands of new chemicals coming into use every year, it is only too clear that any attempt to trace and control all carcinogens, even where deaths have already occurred, would bankrupt the world chemical industry in no time. It is up to individual nations, therefore, to decide what is an acceptable risk, socially and economically.

The ILO Convention, within these limits, requires "every effort" to be made to replace or control carcinogens, all available information to be given to exposed or formerly exposed workers, relevant tests to be carried out on all workers (although there is no provision that the results be communicated to them) and government supervision throughout.

There is also an important requirement that "an appropriate system of records" be kept. Death notifications and employers' records have been instrumental in tracing VCM and Nonox S workers, and record systems are the essence of occupational control where a carcinogen is discovered.

The Recommendation—a less binding formulation for signatories than the Convention—goes much further than the Convention. Most of the extras concern information: the national "competent authority" is to promote epidemiological studies, collect and disseminate all available information, produce "educational guides" for both employers and employees, and try to establish criteria for determining carcinogenicity.

Employers have a duty to seek out information, carry out studies with the aid of the competent authority, notify all affected workers and instruct them regularly. Employers' and workers' organisations both have a duty to carry out information programmes and encourage full participation by workers in control programmes.

The Nonox S story shows how necessary such measures can be, especially in countries (like the UK in the 1940s) with an undeveloped system of safety law enforcement and worker participation.

But implementation could also be a tall order in many countries, and even the UK, which now probably leads the world in health and safety legislation, has confessed that 'it will take a considerable time to set up suitable systems.'

International cooperation on occupational health research is acknowledged to be very poor at present, quite apart from the unsatisfactory distribution of the research effort itself. The ILO's ideas on information could point the way to making use of what information there is. But it seems a pity they are tucked away in a mere Recommendation. ☐

HEALTH and safety legislation in the UK has traditionally been of the Old Testament type—detailed rules with penalties for non-compliance. This had its disadvantages. The Factory Inspectorate, for example, being hopelessly undermanned, was forced into a soft-footed approach rather than waste valuable inspecting time in court taking prosecutions which led to derisory fines even for fatal negligence.

Progress came through the Common Law which enshrines a New Testament-style duty to care for one's neighbour and leaves the practical details to the individual. It was under Common Law, for instance, that two Dunlop employees with bladder cancer were able to obtain damages in 1971 from ICI, which had no legal responsibility to them under the Factories Acts.

Claims for damages have done much to clarify the responsibilities of both employers and employees. Moreover, it was the first successful damages claim for hearing loss induced by noise at work which led to a business boom for the manufacturers of protective equipment—and has made the Department of Employment's code of practice on noise (not statutorily binding) a best-seller.

Purpose-built Old Testament legislation is usually narrow in its usefulness, and in modern industrial conditions may be out of date as soon as the ink is dry. In the UK the new Health and Safety at Work Act, which came into force this year, and unifies the Factory and Alkali Inspectorates into one Safety and Health Executive, goes as far towards the flexible New Testament approach as is possible.

It imposes a slightly more detailed duty of care on employer, employee and supplier. But old detailed regulations built up over a century remain in force, and will be expanded, together with the more recently produced codes of practice.

Replacing the old penalties is a system of improvement and prohibition notices which oblige a manufacturer actually to shut down a process until it is made safe to the satisfaction of the relevant inspector. Arguments about notices are dealt with by the industrial tribunals, leaving the inspector to inspect and hand out notices. This puts a high price on failure to comply with the inspector's recommendations. In serious cases, the executive is free to prosecute on indictment, and the duty of care makes it reasonably clear who is to be prosecuted in each case taken. A jury verdict of guilty will lead to an unlimited fine, or perhaps even to imprisonment.

The picture elsewhere in the world mirrors the unsatisfactory state of pre-1975 UK legislation. Most EEC countries have detailed regulations about the provision of occupational health services in industrial companies. In practice, however, the carefully provided nursing services do little except routine curative work, which leaves out preventive work and epidemiology.

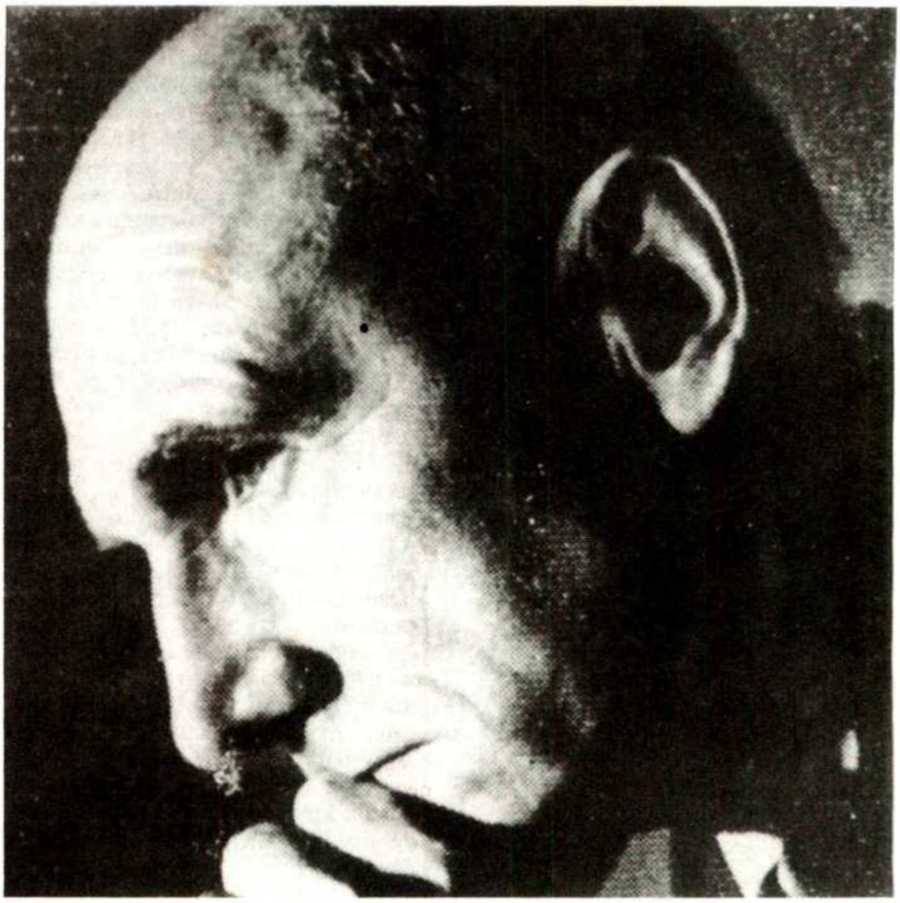
The USA has far-reaching and stringent safety and health laws on the Old Testament line—plus a tiny inspectorate which, it is estimated, is able to visit each factory about once every 400 years.

In the USSR, where there is no clear distinction between employer and employee in industry, the unions lack the sort of power exercised in the UK and the USA. Overall responsibility for safety and health is with government and the unions. Numerous state agencies are also involved —44 of them, for instance, in the development of the Lada (Fiat) car factory under licence from Italy. The result is often confusion and, occasionally, inaction.

Nobel award to Prelog

PROFESSOR Vladimir Prelog of the Federal Technical High School (ETH) in Zürich has been awarded half of the Nobel Prize for Chemistry, at the age of 69, for his work on stereochemistry. It comes to him not so much for any one outstanding discovery, but for a lifetime's work in chemistry which has produced a steady succession of significant researches combining deep thought and elegant experimental work. van't Hoff is considered as the founder of 'Chemistry in space'; Prelog has been the High Priest of this cult for the past 20 years.

Prelog was born in 1906 in Sarajevo in the Austro–Hungarian Empire. He studied in Prague, worked in industry, and then joined the staff of the University of Zagreb in Yugoslavia. Fortunately for Prelog and for chemistry, he found his way to Switzerland in 1941 and joined the great ETH School of Organic Chemistry. His career in Zürich was naturally influenced by his predecessor, Professor L. Ruzicka (Nobel Laureate, 1939), whom he succeeded in 1957. Prelog's structural and synthetic studies on natural products have covered a wide range, including terpenes, steroids, alkaloids and antibiotics.



Vladimir Prelog

A major stereochemical interest started from the preparative work of the Zürich school on medium and large rings, and developed into Prelog's studies on transannular reactions. In the spirit of his time, Prelog not only made new compounds by ingenious methods, but studied their physical properties and their reactivity, using all of the steadily increasing range of tools provided by physical chemistry.

His work in stereochemistry has included a considerable share in the study of conformations, for example, his pioneer work on asymmetric synthesis and the medium-ring work noted here. He delivered one of the earliest Centenary Lectures to the Chemical Society in London in 1949; this significantly influenced the thinking of other pioneers of conformational studies.

Prelog's ability to get to the heart of things is nowhere better illustrated than in the work on the Sequence Rule. This work, the result of a very fruitful partnership between Prelog, the late Sir Christopher Ingold and Dr R. S. Cahn, soon developed into something much more than a method for designating stereoisomers (the "*R* and *S*" system). It led Prelog to consider in a simple, but fundamental way, the geometrical basis of stereochemistry.

The Sequence Rule papers first showed clearly and in a systematic framework of thought the nature of axes and planes of chirality. (The term "chirality", borrowed from Kelvin, has been universally accepted as a convenient term for the property of "handedness".) The need to test the Sequence Rule exhaustively during its development caused Prelog to visualise, and then to make with his students, organic compounds of widely differing types displaying stereoisomerism of kinds apparently unknown in nature, and hitherto unsuspected by organic chemists—for example, cycloenantiomers and cyclodiastereoisomers.

In all of this, Prelog has carried to its logical conclusion the concept of the tetrahedral atom (van't Hoff and Le Bel). The marriage of chemistry and geometry thus arranged by Prelog, and called by him "chemical topology" is already a happy and most fruitful one. All types of stereoisomerism can be derived from the consideration of simplexes in two- and three-dimensional space, alone or in combination.

The general principles were outlined in lectures in Amsterdam, in Dublin, in Hamburg, and in Zürich (Karrer Lecture), and were presented in a more extended from in lectures at Cornell. Some of these ideas have now appeared in a very general paper modestly entitled "Pseudoasymmetry in organic chemistry", which was followed by two detailed papers on compounds showing axes and planes of pseudo-assymetry, giving some experimental 'meat' to cover the elegant geometrical skeleton. The international group characteristically addressed by Prelog in letters as *Stereofreunde* await with great interest and some impatience the fuller story.

Much of Prelog's recent work has been on the borders of chemistry and biochemistry, including his studies on enzymatic reactions. In addition to their supreme biochemical importance, enzymatic reactions provide a suitable terrain for thinking in terms of two-dimensional chirality.

Like many continental professors, Prelog has long had close connections with industry, and he has been for some years a Director of Ciba–Geigy in Basel.

He is a frequent and welcome visitor to laboratories all over the world—and nowhere more welcome than in the UK. Few chemists offer more stimulating discussion to collegues of all ages and interests. He is always one of the liveliest disputants at any meeting, and he was one of the founding fathers of the annual Bürgenstock Conferences on Stereochemistry.

The *Stereofreunde* rejoice in this award to Prelog. Perhaps the pilgrimage to Stockholm will usher in an age of many-dimensional stereochemical thinking.

W. Klyne

international news

US energy policy criticised

by Colin Norman, Washington

IT was just two years ago that former President Nixon coined the term Project Independence as the rallying cry for the drive to make the United States self-sufficient in energy. Since then funds for energy research and development have boomed, the Energy Research and Development Administration has been established to take charge of the effort, and President Ford has reiterated at every opportunity his commitment to the goal of reducing oil imports. Although Mr Nixon's optimistic predictions that the nation could achieve energy independence by 1980 were quickly written off as unrealistic political hyperbole, self-sufficiency is still the guiding principle behind energy policy in the United States.

But, in a report which pulls few punches and which probably represents the most thorough independent analysis so far performed on the federal government's plans for energy research and development, Congress's new Office of Technology Assessment (OTA) has warned that ERDA's strategy is "inadequate" and that it has defects which could even "lead to an increased dependence on foreign energy sources". OTA has raised two broad criticisms of ERDA's programmes, and it has also faulted the agency on a host of specific points.

Prepared by six panels of experts drawn from universities, industry and non-profit institutions, the OTA study concentrated its attention on a massive two-volume plan and programme published by ERDA last June, which will form the basis of the federal government's energy research and development operations over the next few years (see *Nature*, **256**, 83; 1975). It should be noted in ERDA's defence, however, that the plan was published less than six months after ERDA was established, and it was prepared while the agency was recruiting staff and finding its feet. Inevitably, the detailed strategy reflects the sum total of the efforts which ERDA inherited from other agencies, and it's not surprising that the programmes show few significant changes in direction. Indeed, it was no small feat that ERDA managed to produce the plan at all in such a short period.

Nevertheless, OTA's two chief criticisms go to the heart of ERDA's strategy, and they merit close attention. To begin with, OTA said that ERDA's overall plan "is a significant milestone in the evolution of a long-term national energy policy", but it found that the programmes which ERDA is supporting do not "appear adequate to achieve the stated goals". The problem, OTA suggests, is that ERDA has taken too narrow an approach by concentrating almost exclusively on the development of sophisticated technology and neglecting many non-technical factors which could inhibit the implementation of new technologies. Moreover, OTA has accused ERDA of paying insufficient attention to conservation by failing to promote the development of relatively mundane technologies which could reduce energy growth in the short term.

"In addressing the energy goals, ERDA adopted a narrow, hardware-oriented approach," OTA suggests. Although such an approach may be well suited to putting a man on the Moon, or building new weaponry, OTA notes that the energy crisis is "a far more complex and wide ranging challenge". As a result, "ERDA's narrow approach to the national energy policy goals might well fulfill a mission —developing new technology—without providing an answer—a secure energy future. Unresolved 'nontechnical' issues—from inadequate incentives for commercialisation through environmental demands, competitive use of resources, to community resistance—could block the most sophisticated engineering achievement", OTA warns.

ERDA officials are, of course, well aware of such criticisms. When the plan was published last June, for example, Dr Robert C. Seamans Jr., the Administrator of ERDA, stated explicitly that "it is one thing to take a relatively small number of astronauts on an experimental-type mission. It is another thing to put into being in this country an energy delivery system and an energy conservation system which will satisfy the needs of over 200 million people . . . It is a much more complex problem we face than just the research and development".

OTA clearly feels, however, that although ERDA may recognise the need for a broad approach to energy research and development, its plan does not reflect that need. "If ERDA confines its activities predominantly to the proving of the feasibility of technological options, some other entity should address the more complex issues underlying energy solutions," OTA suggests.

A central difficulty, which OTA does not specifically address in its report, however, is that overall energy policy in the United States is hopelessly confused. Congress and the White House have been at loggerheads for months on the question of whether or not oil and gas prices should be decontrolled, for example, and Congress has not yet demonstrated that it has the stomach for taking politically unpopular decisions to curb energy growth in an election year. Energy policy, in short, has become a political football, and swift progress on the "more complex problems underlying energy solutions" should not be anticipated.

As for OTA's more specific complaints about ERDA's plans, the following are among the more important:

- Only about 2% of ERDA's budget is allocated to conservation programmes, yet "successful widespread implementation of conservation programmes . . . can have both a rapid and continuing effect", OTA suggests. ERDA may be neglecting such efforts because that are relatively mundane, and not intellectually as exciting as the development of sophisticated new supply technologies.
- Insufficient emphasis is placed on international cooperation in such areas as nuclear waste disposal, the management of nuclear materials, and the development of ocean resources.
- ERDA is not devoting enough attention to commercialisation of new energy technology. Although ERDA's plan outlines a philosophy for commercialisation, it "clearly needs a more detailed explanation and careful definition of plans for developing a mechanism for coordination with industry".
- "Only limited attention is given to socioeconomic research and analysis in addressing the nation's energy problems". Broadranging research is needed to identify non-technological obstacles to energy solutions.

Conventional wisdom is that electrical energy consumption will continue to grow as a much faster rate than total energy consumption. But OTA notes that "intensive electrification will have a noticeable social impact and may present problems of vulnerability and reliability", and suggests that "the long-term electrification approach should be more thoroughly analysed . . . to make sure that viable alternatives are not lost by default".

OTA's analysis was performed at the request of three Congressional committees which have jurisdiction over ERDA, and which are thus in a sound position to ensure that the agency changes its policies if necessary. The plan will be updated each year and republished in January along with the President's budget request. □

Re-establishment of science office

AFTER months of agitation by prominent spokesmen for the scientific community, the House of Representatives last week approved a bill to reestablish a science policy office in the White House. The Senate is expected to follow suit early next month, and the measure could be on Mr Ford's desk soon after Christmas—almost exactly three years after Mr Nixon decided that he no longer required the services of a full-time science adviser.

The bill swept through the House to the accompaniment of a chorus of laudatory remarks about the benefits which scientists can bring to the heart of national policymaking, and it was eventually approved by the impressive margin of 362 votes to 28. According to Representative Charles Mosher, a senior republican from Ohio who helped draft the measure, the bill also has Mr Ford's wholehearted support.

Scientists are therefore well on their way back to the inner corridors of power, from which they were banished by Mr Nixon in 1973 as the result of a post-election shake up of the White House staff. Mr Nixon dismantled the Office of Science and Technology, which was established by Presidents Eisenhower and Kennedy, and conferred the title of science adviser on the already overburdened shoulders of the Director of the National Science Foundation, Dr H. Guyford Stever.

Although it was fortunate that the science policy apparatus was out of the White House when it was wallowing in the Watergate morass, there have since been numerous calls—including one from the prestigious National Academy of Sciences—for it to be brought back in. As a result, Mr Ford five months ago sent Congress a legislative proposal for a rather modest office, headed by a single science adviser.

The bill passed by the House last week conforms rather closely to Mr Ford's proposal, although a few significant changes were made along the way. The office, which would be called the Office of Science and Technology Policy (OSTP), would be limited almost exclusively to advice and analysis—it would have no direct control over scientific programs or budgets. The director of OSTP would be the President's chief science adviser, and his brief would extend over most of the federal government's scientific affairs, including military technology, the economy, foreign relations, and so on. The budget for OSTP would be about $2 million a year.

As for the manner in which the office would carry out its responsibilities, the bill merely states that the director should "develop appropriate working relationships" with the National Security Council and the Domestic Council and "maintain liaison with" all other units in the President's immediate vicinity. In other words, the office would fit into the White House power structure as best it could.

During the Johnson and Nixon Administrations, the old Office of Science and Technology lost influence largely because of growing disaffection between those presidents and the scientific community, chiefly over the Vietnam War. At the finish, Mr Nixon became agitated because he disagreed with some of the advice he was receiving from the Office, particularly from the President's Science Advisory Committee—a top level independent committee chaired by the science adviser—and he banished the science advisory functions to the bureaucratic hinterlands.

Guyford Stever

After that episode, dismayed leaders of the scientific community who felt, in the trenchant words of Nixon's last science adviser Dr Edward E. David, that "science had been downgraded in national affairs", urged that a more permanent structure be established. The fact that OSTP will be set up by legislation effectively means that it cannot be dismantled simply on a Presidential whim—Congress would have to pass a bill to dismantle it.

The bill now goes to the Senate, where it is being handled by three separate committees. Hearings are already under way, and it is expected that a final version can be produced for a vote by the full Senate in December. The chief architect of the Senate's version is likely to be Senator Edward Kennedy, whose subcommittee on the National Science Foundation has a large stake in the enterprise.

Regardless of the form in which the bill eventually emerges from Congress, speculation is already rife in Washington about who will get the job of science adviser when it is established. According to sources within Congress and the Administration, the leading prospect at present is Dr Simon Ramo, an electrical engineer who is vice chairman of the board of TRW Inc, a major defence contractor. Ramo, who is widely respected in science policy circles, could not be reached for comment last week.

- For a few days last week, worried officials of the National Aeronautics and Space Administration (NASA) tried to determine the cause of an electrical failure which threatened to abort one of the two attempts to land a Viking spacecraft on the surface of Mars next year. A battery, which will be needed during the landing operation and which will also be required to power some of the experimental apparatus on the spacecraft, failed to charge when an attempt was made on October 31. The spacecraft was then about 17.6 million km from Earth. The fault was eventually traced to the charging apparatus, and on November 5, a backup charger was used. Fortunately for the $1,000 million Viking programme, it worked, and NASA officials now believe that the batteries will perform their functions according to plan.

The first Viking spacecraft, whose batteries behaved properly when they were charged last month, is now about 24 million km from Earth. It is expected to arrive at Mars about June 19 next year, and a landing attempt will probably be made on July 4—to coincide with the United States' Bicentennial. □

Canadian diary

from David Spurgeon, Ottawa

THE government of Ontario, Canada's most populous province, has started an interesting experiment in communal technology assessment this autumn. It has invited its people to help it decide directly how to provide for their future electric power needs.

At a time when economic growth is no longer sacrosanct, when consumer groups are growing increasingly critical of the siting of power stations, the placing of power transmission corridors, and the trend towards the adoption of nuclear energy, such a gesture by the province can only be seen as significant.

The method being used to gather the peoples' opinions is the Royal Commission. The Order-in-Council establishing the Royal Commission on Electric Power Planning was approved last July, and public meetings began on October 28. The commission has been instructed to examine the long range power planning concepts of the public corporation (Ontario Hydro) that is charged with providing the power needs of the province, for the period 1983–93 and beyond. It has been told to relate them to provincial planning, to the utilisation of electrical energy and to environmental, energy and socioeconomic factors, and to report, with a list of priorities, on the need for certain Ontario Hydro projects at present under consideration.

The chairman of the commission is a former professor of engineering at the University of London, Dr Arthur Porter. Porter, who now is professor of industrial engineering at the University of Toronto but on leave for a year, has long been interested in the broader implications of science and technology on society. Serving with him are an industrialist (Robert E. E. Costello, vice-president of corporate services, Abitibi Paper Company Limited), a journalist (Madame Solange Plourde-Gagnon, who will represent the consumer viewpoint), a farmer (George A. McCague, who has served on the executive of many farm organisations) and an economist (Dr William W. Stevenson, at present a member of the Ontario Energy Board).

Announcing the formation of the commission, the Provincial Secretary for Resources Development said: "The inquiry may well be the most important in this decade . . . [it] should bring into public focus basic philosophies about much of the kind of life we want for the next century and what price we are prepared to pay for its achievement . . . The public needs to know what demands for electricity will be placed upon Ontario Hydro in the long term, how these needs should be met, and what impact this would have on Ontario's way of life and its physical environment."

During the autumn, the commission will hold a series of preliminary public meetings in Ontario in 16 cities. From these it expects to learn what issues the public wants included in the main inquiry, how and in what format it should proceed, and how it can increase public participation. An attempt will be made, the commission says, "to avoid either an inhibiting or court room atmosphere . . . The preliminary meetings in particular will be very informal."

Some of the major issues and questions expected to arise are:

- Power demands have been increasing by about 7% a year. Should this rate be allowed to continue? What are the chances of its increasing or decreasing?
- How can energy be used more efficiently?
- What are the benefits, costs and risks of alternative ways of generating electrical power? What are the risks associated with nuclear generation, and the environmental implications? How long can we afford to burn irreplaceable fossil fuels?
- How can the important issues associated with land use be decided? Social, economic and environmental factors must be evaluated to decide where industrial and population growth will occur. How should this be done?
- How well are existing procedures working to guarantee public participation in land-use decisions?

One must assume that the hearings will not be equally welcomed by all involved. Recently, Ontario Hydro asked the government for permission to raise its rates by more than 25% because of the rising costs of eletricity production, and promptly found itself under heavy fire from the public and politicians. Before that, lengthy and expensive hearings were held on the subject of the placing of power transmission line corridors. And Ontario Hydro's announced intention to rely increasingly on nuclear power has drawn opposition from various quarters. All this has made the power corporation's attempts to fulfil its mandate more difficult.

Dr Porter says he fears the biggest difficulty will be to get ordinary people, outside the major interest groups, to express an opinion—and in fact to achieve good attendances at the public hearings. A former woman cabinet minister, now heading an inquiry into violence on television, found that only nine people attended a recent public meeting—inspite of what Dr Porter calls a "fantastic press". "If she can't do it," he asks, "how can we?"

The government's move to establish a commission was prompted, says Porter, by a report from Ontario Hydro on their alternative plans for provision of the province's power needs from now until the end of the century. Even assuming the lowest growth rate of power demand considered, the cost came to $20,000 million; if a 20% growth rate were assumed, on the other hand, the cost amounted to $50,000 million. With amounts of money like that involved, the corporation felt it had to draw the public into the planning.

● Another Canadian scientist who is trying to get the public involved in decisions involving science and technology is David Suzuki, a geneticist from the University of British Columbia. Five years ago, he recognised the tremendous potential influence of television, and wrote to the Canadian Broadcasting Corporation (CBC) to suggest that it should carry a television series dealing with science and society.

The result was a locally broadcast programme, Suzuki on Science, and then, last year, a job as host of a new programme called Science Magazine. The show was highly successful, but Suzuki says that because there is no basic constituency for science shows, the CBC planned to withdraw it.

According to Suzuki, the viewers objected: several hundred letters of protest were sent to the CBC, and the show will now return in February 1976 for its second season. Suzuki has also begun this year a weekly series of programmes on CBC radio, called Quirks and Quarks. In fact, he has become so busy broadcasting that he has had to take a year's sabbatical leave.

But Suzuki speaks bitterly about his scientific colleagues' interest in reaching the general public. He is cynical about their motives when they do show an interest in reaching the public, and relates it to their need for research funds for their own projects.

"Basically scientists are public servants", he said in a recent interview about another television programme he hosted, called Earthwatch. "I feel our responsibility is to de-mystify the process [of science] so citizens can participate in setting priorities." He does not portray science as being all good, and attempts to show that the scientist as an individual is a human being with all the feelings of his fellow men. □

Soviet euphoria in wake of Venus probes

from Vera Rich

THE success of the two Venus probes, Venera 9 and 10, the orbital sections of which are still gathering and transmitting data, has led to a spirit of self-congratulation, not only among those concerned with the Venus mission, but throughout the Soviet space programme generally.

This success, coming as it does in the concluding months of the present five-year plan, and in a year already distinguished by the Salyut 4 and Soyuz–Apollo missions, will undoubtedly be a considerable incentive towards increasing the space budget in the next plan

The economic justification for the Soviet space programme, as laid down by the Twenty-fourth Party Congress in 1971, is "to develop long range telephonic–telegraphic communications, television meteorological forecasting and the study of natural resources, geographical investigations and the solutions of other problems of the national economy with the aid of satellites, automatic and piloted spacecraft, and also to carry out fundamental scientific investigations of the Moon and planets of the solar system".

Although basic deep-space research does figure in this list, it seems that a need is felt to justify its importance (and cost) within the framework of the five-year plan. Accordingly, *Pravda* has highlighted not only the political significance of the Venus mission, which illustrates "how rapidly science and technology advances in conditions of socialism", but also the "terrestrial" aspect of the research carried out by the probes—by leading to a better understanding of planetary evolution, they will, it is suggested, facilitate the fuller utilisation of the natural resources of the Earth.

Pravda also carried an article from Professor A. Okhotin, of the Institute of Space Research, on the possibilities of carrying out technological processes in space. This idea has long been of interest to the Soviet space planners—preliminary electron-beam welding and cutting experiments were carried out as long ago as Soyuz 6 in October 1969—and two types of "space technology" are under consideration at present

The first is the construction of large scale orbital space stations, for astronomical and geodetic observations, prospecting, weather forecasting and environmental surveillance. Tentative plans speak of stations with a mass of hundreds of tonnes and crews of several dozen. Such stations could be constructed "most economically" in orbit, and would also require a considerable amount of repair and refit technology for their successful long term operation.

The second type of space technology under discussion involves the processing and production, in conditions of weightlessness and vacuum, of materials which would be almost impossible to produce in terrestrial conditions. The materials would include exotic metal and metal–ceramic alloys, high purity glasses, and large single crystals of semiconductors. The cost involved would, of course, be considerable, but this, says Professor Okhotin, is not the issue. Since the results of the processes would be "unique", he argues, their cost-effectiveness should not be a matter for discussion.

In the Soviet economy, where there is centralised budgetary control and planning, there is, theoretically, no need for any major expenditure to be justified to anyone beyond the higher echelons of Party or Government. Nevertheless, although no costs or estimates of the space programme have ever been published—except for vague hints that the cost of the Lunokhod-bearing Moon probes, for example, is only half that of a manned mission—there has, over the years, been a considerable undercurrent of resentment against the enormous expenditure involved.

Kremlinologists have noticed that a number of 'black' jokes have circulated on the theme of 'pie in the sky', correlating achievements in space with food shortages at home. They note that a 'soft' campaign in the Press is standard Soviet practice in preparing the population for some new government drive or campaign.

This new concentration on the cost-effectiveness of space research seems to suggest that, by forestalling possible criticism, next year's Party Congress will include in its "Theses" for the coming Five-year Plan a considerable extension of the Soviet space programme. □

Reprocessing 'scare' in Britain

by Allan Piper

IF a backbench motion at present circulating around the Houses of Parliament gains sufficient support, the reprocessing of spent nuclear fuels in Britain may well be an issue to come under the scrutiny of the Select Committee on Science and Technology during the next Parliamentary session The matter came to the fore a couple of weeks ago when some newspapers raised a furore over negotiations which could result in the transport of irradiated Japanese nuclear fuels to Britain for reprocessing at Windscale in Cumbria.

The Parliamentary moves may well have arisen directly from that publicity. But so far the initial press hysteria has signally failed to provoke any other reaction of consequence, and even the 80 or so Members of Parliament who have already signed the motion—and who, according to some reports, can expect wide ministerial support—have chosen a sensibly judicious response to claims that Britain is in danger of becoming a "nuclear dustbin".

Most informed observers are agreed that such misplaced concern can only have sprung from a complete ignorance of both the probable terms of the eventual contract and the overall picture of the British nuclear industry. The deal, which could be worth £300–500 million to Britain, depends on Japanese agreement to take back an amount of waste equal to that produced by the reprocessing.

It is likely also that the British Government will insist that the contract include a clause releasing British Nuclear Fuels (BNFL), which owns the Windscale plant, from its obligations should it fail to develop a successful technique for vitrifying the radioactive waste.

So far the Japanese have shown no signs of baulking at those conditions, which effectively mean that even after the deal has gone through Britain will be left with no 'extra' waste. She will not in any case have to accept Japanese material unless a safe way of transporting its products is developed.

Moreover, the quantity of material which the Japanese will send to Britain—4,000 tonnes will be delivered to Windscale during the decade following 1979—is hardly significant even by comparison with the 1,500 tonnes already reprocessed annually in the UK. (A considerable proportion of this, incidentally, comes from British designed, first generation magnox reactors operating in Latina in Italy and Tukai Mura in Japan.)

Even if the quantity of Japanase material is considered on its own, the figures are rather misleading to the uninitiated: of the incoming 4,000 tonnes of irradiated fuel only some 3% eventually winds up as the potentially dangerous, waste fission products which have aroused the press. Of the rest, about 95% comes out as depleted uranium—which has virtually no radioactivity—and less than 1% becomes fissile plutonium, itself a useful nuclear fuel.

As both of those latter products have some utility—depleted uranium, which is extremely dense, is fast replacing lead in many areas of technology—it seems likely that the Japanese will willingly repossess them along with the waste itself. So, all in all, there will be very little foreign material remaining in the "dustbin".

There are other reasons, quite apart from the statistics, why Britain should wish to undertake the reprocessing of foreign nuclear fuels. First, there is a strong political argument. The Japanese approached BNFL in the first place because their own government will grant licences to operate nuclear plant only if provision can be made to reprocess irradiated fuel. Like virtually every other developed nation Japan is now committed to the development of nuclear power as an energy source, at least for the next couple of decades.

In those circumstances there is really very little reason why Britain, which not only has almost 25 years of valuable and highly regarded experience in the reprocessing field but is also a signatory to the Non-Proliferation Treaty, should discourage a move which ensures that Japan does not develop the wherewithal independently to obtain plutonium and, thus, nuclear weaponry.

There are also sound financial reasons why Britain should continue the negotiations. The fuel which the Japanese hope to send to Windscale will come from American designed boiling water and pressurised water reactors. Like the latest generation of British reactors, these use uranium oxide rather than metallic uranium. Although the existing facilities at Windscale can reprocess only the latter type of fuel, used by magnox reactors, it is expected that the deal will include a Japanese downpayment of at least £100 million towards the cost of a new £300 million oxide reprocessing plant that Britain will in any case need for her own requirements in the 1980s.

Meanwhile, the final outcome of this latest controversy over what has become something of a hardy perennial in Britain remains to be seen. ☐

Votes in sewage?

KENNETH MELLANBY

OUR politicians complain of public apathy. Meetings held during the last General Election were usually badly attended In many country districts even the appearance of a Cabinet Minister only produced a handful of listeners, and the platform party often outnumbered the audience. It was surprising to find recently in the Huntingdonshire village of Warboys that when the Parish Council called a meeting to discuss the proposal to turn a clay pit on the edge of the village into a dump for toxic wastes, more than 500 people representing every section of the population, turned up.

Concern for conservation of the environment is usually a middle-class, rather elitist preoccupation, and the mass of the population is either apathetic or hostile Attempts to save ancient buildings, even those of sufficient merit to be "listed" by the Department of the Environment, may be criticised by local councillors who state that these ruins would be better pulled down and replaced by supermarkets, bingo halls or public conveniences. There is little support from all but a minority for setting up nature reserves. It is generally believed that there is little support for spending public funds on such activities as improved sewage works The Warboys meeting suggests that this last assumption may be wrong, and that there may indeed be votes in sewage.

The Warboys affair is a complicated story The London Brick Company operates a small works there, using clay dug out of nearby ground. This has produced a deep pit covering about 30 acres. Such holes are now valuable, as our affluent society finds it increasingly difficult to get rid of many types of waste. London Brick has several other holes, and operates a waste tip in Bedfordshire under the somewhat significant trade name of "Easidispose". The company applied for planning permission to use the Warboys pit for the disposal of refuse, including poisonous wastes This was opposed by the Parish Council, but it was overruled by the County Planning Committee which gave planning permission, though only under stringent conditions aimed at protecting the local environment.

The people of Warboys were not satisfied. Like other citizens of the old county of Huntingdonshire, which was engulfed in Cambridgeshire on April 1, 1974, they were suspicious of the new authority, and complained that they were being used as a dump—no similar scheme existed in Cambridgeshire, which sent its toxic wastes to Pitsea in Essex. Notwithstanding assurances from the county staff, they were not convinced that the dump would be safe. The information provided by London Brick about the substances to be dumped gave them little reassurance, for the list resembled the answer to a parliamentary question prepared by a wily civil servant, in that it was true but at the same time said almost nothing. It gave full details of comparatively harmless chemicals, and then such meaningless information as "sludges from wash-pits" and "heavy metals".

The meeting was addressed by county staff and London Brick officials. I was myself involved at the request of the Parish Council. Having heard of all the precautions to be taken, I asked the company's spokesman if he could give an assurance that the tip would never harm the environment of Warboys. I added that there was cause for concern, as I had visited the Bedfordshire Easidispose dump, having been told by villagers living nearby that it made their life a misery. I said that the dump stank, had poor security and so was a danger to trespassing children—would that at Warboys be better? Very honestly, an official of London Brick refused to give me the assurance I sought. He also reproved me severely, saying that he was surprised that someone of my standing had not approached his company so as to be shown what really went on in its dump. This brought the house down.

For three hours the villagers questioned the officials and the company's representatives. Although the answers were frank, and answered many of the specific worries, the audience remained hostile. When the Chairman of the Parish Council put the matter to the vote, not one single citizen supported the motion that they were satisfied with what they had heard. They all agreed to continue their opposition to the establishment of the dump.

There are lessons to be learned from this affair. We all want to get rid of our waste—but only in some one else's parish. Are we doing enough to reduce the problem, and should we be recycling more materials even when this does not seem to be economic? The main lesson, however, is that the ordinary people *are* concerned about some factors in their environment. If those of us who have been involved with the less popular topics such as old buildings and wildlife can show that we are equally concerned with mundane issues like refuse dumps, then we may be able to recruit a much wider section of the population into the conservation movement. We need them if we are to preserve our, and their, environment.

correspondence

Stantec Zebra

SIR,—May we venture to answer your question (October 16, page 544) regarding the present whereabouts of the Stantec Zebra? It is alive, well and working unconscionably hard somewhere approximately 350 miles north of St Albans.

The Computer Board has little reason for complacency as regards the implementation of the Flowers report at Edinburgh Regional Computing Centre (ERCC). Subsequent to the return of a rented IBM 370/158, the centre finds itself without effective access to a major batch processing facility, with concomitant serious consequences for teaching and research at the Universities concerned (Edinburgh, Glasgow and Strathclyde).

The expensive and wasteful débâcle ensuing from totally inadequate attempts by the Computer Board to provide comparable facilities on an IBM 370/168 situated at Newcastle University defies comprehension.

Yours faithfully,

D. WHITE P. MALLISON
C. GILMORE D. MACNICOL
R. EMANUEL A. F. CAMERON
K. TYLER

University of Glasgow, UK

Mosquitoes

SIR,—Though not directly connected with the Research Unit on Genetic Control of Mosquitoes in New Delhi, I have followed closely the progress of its research work and the recent unfortunate controversy which surrounded it. I would like to comment briefly on some of the points raised in the letter (September 18) by Mr C. Raghavan and Dr K. S. Jayaraman of the Press Trust of India on the subject of the research unit:

- The release strategy, as set out in the unit's protocol for the planned experiment in the town of Sonepat, was to adjust the numbers of sterile mosquitoes to be released in relation to the number of wild mosquitoes currently emerging in the town, so that any females unavoidably released would constitute only a small minority of the total female population. The journalists' mention of the release of "at least 2,000 females a day" makes the unwarranted assumption that the unit's maximum possible output of 400,000 males a day would have been required in this experiment. In reality, during the first week of the experiment 35,000 sterile males were scheduled for release daily and these would have been unavoidably accompanied by about 90 females.
- The releases in Sonepat would have been from a grid of over 500 release points in the town. All mosquitoes for release were to have been marked with the same colour. Therefore, among the samples of mosquitoes recaptured there would have been no way of knowing from which of the release points or which day's release they originated. Can the journalists seriously suggest that a specialist in mosquito ecology could 'extrapolate' such data to give information on the dispersal pattern of female mosquitoes which would be of use to someone interested in biological warfare?
- It is typical of the journalists' method of argument that in support of grave allegations against the work of the WHO on mosquitoes in India, they produce an unsubstantiated allegation about the work of the FAO on sugar in Cuba.
- The journalists refer to "bird migration studies supoprted by the GCMU [*sic*] and the US Army". In fact the research unit in Delhi had no connection whatsoever with the bird migration study.
- The Assessment of the National Filaria Control Programme (India) 1961–1970 (C. G. Pandit, Indian Council of Medical Research, 1971), recommended a dual strategy based on vector control and drug treatment, contrary to the journalists' statement that exclusive reliance on drugs was advocated. The research at the Filaria laboratories of the Indian National Institute of Communicable Diseases reflects this dual strategy.
- The journalists have missed the point about the conclusion that can be drawn from the history of the eradiction of *Aedes aegypti*, but not other *Aedes* species, from Poona. It has never been suggested that this disproves the existence of a degree of cross immunity between dengue antibodies and yellow fever virus. What it does provide is a practical demonstration that if *A. aegypti* (the vector of urban yellow fever) is removed from an Indian town, this does not cause the appearance of yellow fever, contrary to the fears expressed by the journalists about the consequences of the unit's planned experiment in the town of Sonepat.
- I am surprised by the journalists' opinion that research on "sophisticated technologies" should be restricted to the developed countries, as this runs directly counter to the commonly expressed view that the developing countries require more home-produced technology which has been tested for its feasibility and developed in relation to the actual field conditions in which its application is required. Fortunately the journalists' attitude is not widely shared, and the recent successful genetic control of an *Anopheles albimanus* population in El Salvador (Lofgren, C. S., *et al., Am. J. trop. Med. Hyg.*, **23**, 288–297; 1974) suggests that these techniques may provide a practical weapon in the struggle against malaria and other vector-borne diseases on a much shorter time scale than that suggested by the journalists.

Yours faithfully,

R. J. WOOD

University of Manchester, UK

A hundred years ago

MR GLADSTONE AT GREENWICH

WE may surely regard it as a hopeful sign of progress that a whole page of the *Times*, as well as of other daily papers, of last Friday was practically devoted to the discussion of matters connected with Science and Art. When we find the daily papers giving so great prominence to these subjects, and when men of such position and mark as Prince Leopold, Mr Gladstone, and the Lord Chief Justice, take what is evidently a genuine interest in the progress of science and art education among the lower classes, it seems quite safe to infer that this movement has at last taken a prominent and important place in the everyday life of the country.

Prince Leopold, in his address at Oxford, showed that he had taken some pains to become acquainted with the latest statistics of the Science and Art classes. The discrepancy between Prince Leopold's statistics and those of Mr Gladstone has been commented upon in the *Times*. At the same time it is gratifying that a man like Mr Gladstone should think it worth his while to take an interest in the matter at all, but when he does determine to think about it, the least he can do is to inform himself correctly as to statistics.

From *Nature*, **13**, 41, Nov. 18, 1875.

news and views

Chromosomal aberrations as tests for mutagenicity

from John R. K. Savage

THE very obvious structural changes which can be induced in chromosomes, and the relative simplicity of the cytological methods which render them observable have made 'chromosomal aberrations' a popular criterion for the evaluation of potential environmental hazards.

Recent developments of staining methods to detect sister chromatid exchanges, SCE (a reciprocal exchange of segments at homologous loci between the two chromatids that comprise the metaphase chromosome arm) have now added another group of structural changes, the induction of which may be utilised as a screening test for mutagens and carcinogens. Results with a wide range of agents reported by Perry and Evans in this issue of *Nature* (page 121) indicate that the test is more sensitive, quicker and easier to use than those employing conventional aberrations. It may also prove to be more relevant since SCE are produced during the DNA synthesis phase, a time when it is thought that most mutations are fixed or established (Auerbach and Kilbey, *A. Rev. Genet.*, **5**, 163–218; 1971).

Perry and Evan's study of SCE illustrates a number of areas where there is a need for caution if the occurrence of induced aberrations is to be used as an indicator of genetic damage.

Of first importance is understanding the nature and mode of action of the agent used to produce aberrations. The properties of the substances used by Perry and Evans covered a wide spectrum. Some had a short half-life giving an acute dose, others were continuously active throughout the treatment period, and one other (cyclophosphamide), because it requires metabolic processes to convert it to an active mutagen, is ineffective *in vitro*. Such information is vital for designing the experimental test.

The need for parallel *in vivo* tests is indicated, not only because of metabolic activation but because the body also has considerable powers of detoxification (Williams, *Detoxication mechanisms*, Chapman Hall, London; 1959). It is possible to expose chromosomes *in vitro* to substances in a form, concentration and *p*H which would be impossible *in vivo*. Moreover a range of different cell systems is needed. Inferences on the mutagenicity of maleic hydrazide based solely on work with plant cells would have been most misleading.

Second, there is a need to appreciate the time of action of the agent in relation to the cell cycle. The majority of chemical substances which produce aberrations, although probably able to induce lesions at all stages of the cycle, require that the cell pass through one or more DNA replication phases in order to allow the lesions to mature into structural chromosome changes. At the same time, these substances invariably cause considerable dose-dependent perturbations in, and delay of passage through, the cycle. Factors such as these will determine how soon after treatment and with what frequencies aberrations will be observed in the test cells. Mitotic perturbation can lead to the simultaneous presence of cells in first, second and even third mitosis after treatment, at the time of sampling, and it may not always be possible to distinguish between them (particularly in the presence of a persistent mutagen) or to assess the effects of cell selection and secondary (derived) aberrations. It is clear that no one 'standardised' experimental design will serve for all mutagenic agents.

Undoubtedly the biggest problem encountered in aberration work is that of quantitation. Given accurate classification and recording of aberration types, can an interpretable dose-response curve be constructed?

On the one hand there is the difficulty of determining the effective 'dose' received by the vital targets in the cell. This may or may not bear a simple relationship to the molar concentration added to the environment of the cells, for it is conditioned by such things as the nature of the agent, the form in which it is supplied, its mode of uptake by the cell, and the density and mitotic status of the test cell populations. If the agent is unstable, the difficulties are enhanced.

On the other hand, there must be some unique quantity or 'yield' to set against dose. Different yields in different cell cycle compartments (see Fig. 2 of Perry and Evans) alert one to the fact that sensitivity to the agent may change as the cell progresses towards mitosis. In passing it should be noted that such changes are not the same for all types of aberrations. Since the cells of a population do not progress at a uniform rate through the cycle, it follows that samples of metaphases, examined at different times after an agent is introduced, comprise mixtures of cells with differing sensitivities and a corresponding variation in observed mean yield is to be expected. The profile of the variation of yield with time of sampling is influenced by many factors, in particular mitotic delay and perturbation (Savage and Papworth, *J. theor. Biol.*, **38**, 17–38; 1973). All known aberration-inducing agents perturb the mitotic cycle, the degree of perturbation differing with dose.

In these circumstances, a unique yield for a given dose does not exist, so that a dose response curve can take a variety of shapes depending on the sampling methods and times used. A great deal more detailed mathematical work on the kinetics of disturbed cell populations is required before any significance can be attached to the form of such curves.

A fact often overlooked when structural aberrations are used as a test system is the very poor resolution they afford in molecular terms. It is all too easy in discussion to couple molecular damage (such as DNA strand breakage or cross-linkage) and observed chromosomal changes, forgetting size differences of many orders of magnitude. Using the new chromosome banding techniques, the loss of a chromatid band (or a major part of it) is about the limit of aberration detection with light microscopy. The Paris Conference 1971, banding norm (*Birth defects*, **VIII**, **7**, National Foundation, New York) has 1,200 chromatid bands in the diploid human female karyotype. If we give equal weight to each band and distribute the 5.8 pg of DNA (Abrahamson *et al.*, *Nature new Biol.*, **245**, 460–462; 1973) uniformly between them, then each band contains 1.61×10^6 nm or 4.73×10^6 nucleotide pairs (a scale model in cotton thread would require ~ 200 m). Thus many significant structural changes could take place within a band and remain wholly undetected by visual means. Equivalence of visible 'breaks' and molecular lesions is therefore of very doubtful value. The rationale for using aberrations is based on the premise, probably valid, that if a substance induces visible change (much of which is lethal to the cell that carries it) it will also induce invisible change having a high probability of transmission and of genetic consequence. Absence of visible changes however does not imply that there are no invisible ones.

Finally, it is worth considering the nature of SCE in relation to conventional aberrations. The former are all reciprocal and symmetrical (that is, they do not give rise to dicentric chromatids and acentric fragments) complete (no loose 'ends') and, within the resolution limits discussed above, the exchange is confined to homologous loci. This is true also of the occasional chromatid interchanges ('quadriradials') found in Blooms syndrome where SCE is much elevated (Chaganti *et al., Proc. natn, Acad. Sci. U.S.A.*, **71**, 4508–4512; 1974). By contrast, conventional chromatid aberrations, whether produced by chemicals or radiation, are frequently asymmetrical, often incomplete and certainly not confined to homologous loci either within or between chromatids (Savage *et al.*, *Chromosomes Today*, **4**, 267–276; 1973).

That SCEs constitute a highly sensitive test, and that agents differ in their ability to induce them is now established beyond reasonable doubt, but in the light of the above differences it would seem unwise to substitute them completely for tests using conventional aberrations until more is understood of their relative genetic significance. Both would seem to be necessary at present.

The observations that with certain treatments, chromosomes may be 'shattered' into small discrete segments (Lovelace, *Genetics*, **38**, 675–676; 1953), that chromosomes precociously condensed in S phase appear to be fragmented (Johnson and Rao, *Nature*, **226**, 717–772; 1970) and the increasing evidence that DNA/protein subunits of uniform size exist in eukaryotic chromosomes (McGhee *et al. Chromosoma*, **52**, 189–205; 1975) lead one to speculate on the doctrine of chromosomal integrity during interphase. Might it be that enhanced SCE implies some interference with a mechanism which links chromosomal subunits? Further studies of these aberrations may throw some new light on the vexing question of chromosome structure and organisation. ☐

Organising neural development

by Miranda Robertson

In the experiments of Chung and Cooke, reported on p. 126 of this issue, two lines of enquiry converge on the optic tectum of *Xenopus*, the African clawed toad. The consequence is that the classical embryological concept of specialised organising regions in development can for the first time be brought to bear directly on the specific question of how ordered neuronal connections are set up in the central nervous system.

Embryonic organisers, according to Spemann, who developed the concept in the early years of this century, are specialised regions which control the morphogenesis and differentiation of the tissues immediately surrounding them. The classical organiser has recently undergone a renaissance in the light, in particular, of the positional information theory due to Lewis Wolpert, and it now emerges as the source of a morphogenetic signal providing information on the relative positions of the cells in the developing field. At the earliest stages of development, the entire embryo is a single field within which the positions of cells are defined by one organiser. According to positional information theory, the subsequent development of the embryo depends on its interpretation of the positional signal. As part of the process of differentiation, the cell acquires a permanent positional label: if it is moved after that stage, it will differentiate in a manner appropriate to its original position.

There is however a stage before the cell's positional label becomes permanent during which the tissue as a whole retains the ability to regulate. If some of the cells are removed at that stage, the remainder adjust to form a complete tissue (or at very early stages, a complete embryo).

It is within this framework that neurobiologists have been attempting to understand the development of the amphibian retino-tectal projection. It has been known since Sperry's classical demonstration in the early 1940s that rotation of an amphibian's eye after section of the optic nerve is followed by the re-establishment of optic connections in the tectum in precisely the same order as before, so that the unfortunate amphibian's visual world is rotated by the same number of degrees as the eye, as his misdirected reflexes bear witness. More recent experiments have done much to explain when and how the retinal cells acquire the information that enables them to reconnect in this invariant way.

The principle of these experiments is to change the position or the orientation of the eye at various stages of embryonic development and to use the retino-tectal map of the adult (mapped electrophysiologically) to assay the effects of the operation. Thus by rotating the eyes of *Xenopus* larvae at different stages, Marcus Jacobson (*Devl Biol*, **17**, 202; 1968) made the important discovery that the cells acquire fixed positional labels along orthogonal axes which are specified at slightly different stages of development. The antero-posterior axis is irrevocably determined by stage 29; the medio-lateral axis a little later at stage 30 or 31.

More recently, Jacobson and Hunt have found evidence (*Proc. natn. Acad. Sci., U.S.A.*, **20**, 507; 1973) that the information for specifying these coordinates derives from a positional signal operating over the entire animal. They have transplanted eyes to the animals' flanks until after the critical stages and then replaced them in the orbit, to show that the retino-tectal map is normal provided that the orientation of the eye was unchanged with respect to the major axis of the animal during its sojourn in the flank. These results conform to modern theory in the sense that the positional signal apparently conveys only general spatial information and differentiation depends on the cell's interpretation of it.

But how specific is that interpretation? Sperry originally postulated two sets of precisely matching chemical labels in retinal and tectal cells. The experiments of Michael Gaze and his collaborators over the past few years however have shown that if such labels exist at all they must remain labile. The most compelling recent evidence is based on the geometry of retinal and tectal growth. The retina grows in concentric rings, whereas the tectum extends backwards from its anterior lateral pole. In spite of this disparity in the geometry of growth at the two surfaces, the orderly array of neural connections is maintained throughout the different stages of larval development (Gaze *et al., Proc. R. Soc., Lond*, **B185**, 301; 1974). Gaze and his colleagues suggested on the basis of their observations that the connections continually shift during development, and their suggestion has now been confirmed (Chung *et al*, *Proc. R. Soc.*

Lond., **B187,** 449; 1974).

Clearly there is no unique cell-to-cell matching, and Chung promulgates the view (*Cell*, **3,** 201; 1974)—credited to Lettvin—that what the retinal cells possess is information on their position relative to each other. If that is rigorously preserved, all that is needed at the tectum is an axial polarity to orient the entire array of retinal fibres.

It is the development of that axial polarity that Chung and Cooke have now subjected to experimental scrutiny. The aim of the experiments was to rotate the tectum at different stages of embryonic development and by later mapping the retinal projection, to determine the stage at which its axial polarity became fixed—in a manner precisely analogous to Jacobson's experiments on rotation of the retina.

The operation is technically very difficult because of the size and fragility of the embryonic brain and because at the stage at which most of the operations were performed (21-24) the different brain regions are determined but not yet anatomically distinct. The rotated tissue in each case was of the order of 15 cells square, and in the light of later anatomical analysis, seems sometimes to have included part or all of the adjoining diencephalon, and/or to have excluded part of the tectum.

Anatomically, therefore, the consequences of the operation varied considerably. Because the brain regions at this stage are determined but not yet differentiated, disturbed fragments of diencephalic or tectal tissue regulated to produce complete structures, giving rise to duplicated diencephalons or tecta, or both, at various positions depending on the exact origin and extent of the rotated tissue.

The effect of the anatomical chaos upon the retino-tectal map however turned out to be strictly independent of the orientation or morphology of the tectal tissue itself. The electrophysiological results clearly showed that the axial polarity of the map was determined by the diencephalon. Where the diencephalon was in its normal position anterior to the tectum, the map was normal; where it was transposed so that the tectum was anterior to the diencephalon, the map was reversed. In one case, where a tectum was wedged between two diencephalons, two maps with opposite polarities were recorded, one starting from each tectal edge. Morphological aberrations were ignored by incoming optic fibres. In an animal in which two tecta had developed side by side, the optic nerve entered aberrantly between the tecta but nonetheless spread out over the surfaces of both to give a normally ordered and polarised map.

Chung and Cooke infer from these results that the diencephalon acts as an organiser controlling the axial polarity of the retino-tectal projection, and preliminary results from grafts of diencephalic tissue alone seem to support this conclusion. They also raise the question of whether the tectal cells ever acquire permanent positional labels.

But the results raise numerous other important questions which are now accessible to experimental investigation. One of the most fundamental concerns the nature of the diencephalic signal. Morphogenetic signals have so far proved uniformly elusive, but until now, neurologists have not known even where to look for them. A further fascinating question is whether it is the same signal that first determines the morphological polarity of the embryo and later provides the axial polarity that guides the optic fibres. The morphological determination of the brain, the determination of axial polarity in the retina, and the determination of the axial polarity of the tectal map are all clearly separated in time.

But the two known organising regions in *Xenopus* are very close neighbours in space. The diencephalon develops from a region overlying the blastopore lip, which is the classical organiser for the early stages of development.

Could the classical and diencephalic organisers control all three developmental processes with the same signal, interpreted by the cells in different ways at different times? The only evidence so far for temporal changes in the interpretation of an organising signal comes from work on the cellular slime mould (Rubin *et al.*, *J. Embryol. exp Morph.*, **33,** 227; 1975), which has the heuristic disadvantage that it is not an embryo but the unique advantage of possessing a chemically identified morphogenetic signal. The identification of an organising region in the amphibian brain offers the opportunity of investigating this question in the developing nervous system of a vertebrate. □

Glassy polyethylene

from P. D. Calvert

In principle any crystalline material can be converted into a disordered glassy solid if it is cooled sufficiently rapidly from the molten state. For some time it has seemed that polyethylene crystallised too quickly for the glassy state to be reached even by splat cooling techniques. But recently Hendra and co-workers have reported the preparation of glassy polyethylene simply by dropping a foil-covered film of polyethylene at its melting point into liquid nitrogen (*J. Polym. Sci. Lett.*, **13,** 365; 1975). The infrared spectrum shows it to be amorphous at 120 K and crystalline after warming to 180 K. Other workers must apparently have allowed their samples to warm up before studying them.

There is no shortage of polymers which can be produced in both crystalline and glassy states but polyethylene is by far the most studied synthetic polymer and there is already controversy over its glass transition temperature. As normally prepared polyethylene is semicrystalline containing 30–70% amorphous material dispersed between small crystallites. The glass transition temperature of this amorphous phase has been claimed to be 125, 193 or 240 K. In a 1973 review (*Macromolecules*, **6,** 288) Boyer argued strongly for 193 K. Hendra's experiments show crystallisation occurring above $\sim$ 170 K suggesting that the glass transition must be below this.

At the Shrivenham meeting of the polymer physics group of the Institute of Physics, Hendra discussed some even more controversial results. Using a peak in the Raman spectrum at about $20\,\mathrm{cm}^{-1}$, which is a longitudinal acoustic mode of the whole chain segment within a crystal, they could measure crystal thicknesses. Crystals which form at 160–180 K have a thickness of about 170 Å parallel to the chain. This value is similar to that found in high temperature crystallisation. Since chain motion must be very restricted so close to the glass transition this result suggests that large scale untangling is not necessary for crystallisation and so throws doubt on the chain-folded crystallisation concept which is the basis of most present theories of crystallisation.

Worse still, they found that the crystal thickness formed on warming the glass depended on the temperature at which the polyethylene was melted before quenching to the glassy state. Melting at 400 °C gave rise to 155 Å crystals; at 160 °C the crystals formed were 180 Å thick. This is heretical in that liquids are not thought to contain any significant structures which could be quenched in and affect subsequent crystallisation. The results might be attributed to degradation of the polymer or destruction of nucleating particles but melting at 160 °C subsequent to melting at 400 °C again produces 180 Å crystals so the effect is reversible.

Thymus-dependent lymphocytes

from P. B. Medawar and Elizabeth Simpson

Under the patronage of the Rockefeller Foundation a conference was held at the Villa Serbelloni, Bellagio, Italy, from September 1–6, to consider the functional taxonomy of populations of thymus-derived lymphocytes, and by implication their role in the functional integration of the immune system.

THE subjects under discussion included 'T cell differentiation and subsets of T cells,' 'What do T cells see?' 'Ir control and cell interactions,' 'Suppressor T cells,' 'Idiotypes and networks,' and 'The IXth linkage group of genes in the mouse.'

Although the identification of Ly differentiation surface markers for peripheral T cells (Boyse *et al.*, *Proc. R. Soc.*, **B170**, 175; 1968; Cantor and Boyse, *J. exp. Med.*, **141**, 1376; 1975) was seen as a significant step forward in characterising functionally distinct subsets of T cells, the limited availability of anti-Ly sera is an obstacle to the general application of a system of classification based solely on such surface markers. On the basis of the published information referred to above, and unpublished information provided by several participants (E. Boyse, H. Cantor, P. Beverley, D. Schendel and K. Rajewsky) a tentative classification table was nonetheless drawn up taking into consideration the characteristics of three basic peripheral T cell types, T helpers (T_H), T cytotoxic cells and T suppressor cells ($T_{C,S}$) and early or immature T cells (T_E). The scheme (Table 1) attempts to bring together a number of older observations with the new data obtained from experiments with anti-Ly sera.

In answer to 'What do T cells see?' it is possible that T cells are triggered by antigen associated with molecules of the major histocompatibility complex (H molecules). Three pieces of evidence are particularly relevant.

(1) In some systems cytotoxic T cells seem to recognise a neoantigen formed by association between hapten-like determinants and H-2K and H-2D molecules (see Lennox, *Nature*, **256**, 7; 1975).

(2) Ly 1$^+$ helper T cells (which are active in both antibody and cytotoxic responses) probably react to Ia determinants, coded by the I region of the major histocompatibility complex, while Ly 2, 3$^+$ killer cells respond mainly to H-2K (Lipsky and Rosenthal, *J exp. Med.*, **141**, 138; 1975; Cantor and Boyse, *J. exp Med.*, **141**, 1390; 1975).

(3) Several studies suggest that most, if not all T cells have receptors for H antigens (for example, Ford *et al*, *J. exp. Med.*, **141**, 681; 1975).

For triggering to occur, therefore, the T cell receptor may bind to a complex formed by H molecules and specific antigen. Either, one receptor site may bind the neoantigen, or two separate binding sites (on the same or different molecules) may recognise the self H antigen and the foreign haptenic determinant. More specifically it may be that antigen associated with H-2K/H-2D molecules would preferentially trigger an Ly-2,3$^+$ killer or suppressor cell response, while antigens linked to Ia molecules would preferentially stimulate Ly-1$^+$ helper activity. This model implies that the primary function of T cells is to "screen" self H molecules for the presence of non-self determinants. Further work is needed to isolate the T cell receptor and/or the proposed antigen–H complexes before this hypothesis can be rigorously tested.

Table 1 T cell subsets

Character	T_H	Subset* $T_{C,S}$	T_E
Ly phenotype	1	2,3	1,2,3
Ontogeny early	+		+
Primary CMI reaction (to H-2)	"LD" (I region)	"SD" (K,D regions)	
Cytotoxic cells:			
Precursors		+	
Effectors		+	
T/T help			
Non-specific (MLR)	+		
T suppression (probably T/T):			
Specific		+†	
Non-specific			+ ?
T/B help: specific	+		
Ia phenotype		+ ?	
Histamine receptor		+	
ATx sensitivity			+

+, Character present.
+ ?, Character probably present.
*It is not implied that all cells in a given subset exhibit all the characters listed.
†Ly 2 positive

The way in which I region immune response genes exert their control over cell–cell interactions in immune responses, and hence over both T and B cell responses to specific antigen, is still a matter for debate. There are at least three interactions which must be considered—macrophage/T, T/T and T/B. A requirement for I region identity for these interactions has been shown in some systems (Katz *et al.*, *Proc. natn Acad. Sci. U.S.A.*, **70**, 2624; 1973) but not in others (McDevitt *et al.*, in *Genes, Receptors, Signals*, 597, Academic Press, 1974; Heber-Katz and Wilson, *J. exp. Med.*, **142**, 928; 1975; von Boehmer *et al*, *J exp. Med.*, **142**, 989; 1975). Genetic analysis of the antibody response of different strains to several antigens, Thy 1, LDH, GLΦ and TGAL has demonstrated complementation groups, indicating that at least two genes are involved (Zaleski *et al.*, *Trans. Proc.*, **5**, 201; 1973; Melchers and Rajewsky, *Eur. J. Immun.*, in the press; Dorf *et al.*, *J. exp Med.*, **141**, 1459; 1975; Rude and Gunther, *Progress in Immunology II*, **2**, 223; 1974; Munro and Taussig, *Nature*, **256**, 103; 1975). For LDH and GLΦ the two genes are encoded in different I subregions of the major histocompatibility complex, while for TGAL both genes may be in the IA subregion. In the response to GLΦ there is some evidence that both genes are expressed on B cells, whereas for TGAL there is evidence that one gene is expressed by B cells (± macrophages) and the other on T cells. The simplest view would be that most T cell interactions with other lymphocytes and macrophages are mediated by various proteins bearing different Ia specificities all of which are under Ir control.

The ways in which T cell suppression controls immune responses are still unclear, but recent work has clarified at least some pathways. It has been suggested that an antigen may have different determinants for initiating help and suppression, but that these determinants must be linked on the same molecule.

One very striking finding has been that in several quite different systems, for example antigen-specific suppression, allotype suppression and con A induced suppression (Feldmann *et al.*,

Stanley rides again

by Mary Lindley

BIOLOGISTS who joined the recent Zaire River expedition brought back a fine collection of flora and fauna, even if at times they may have felt that the price was rather high. In spite of bothersome details such as malaria, amoebic dysentery, fearsome rapids and mysterious footprints, the report delivered by three members of the expedition to a meeting of the Linnean Society on October 16 indicated a mission largely successfully accomplished.

Conditions were considerably better for the scientists, soldiers and other experts who set out under the auspices of the Scientific Exploration Society, in October 1974, than they had been 100 years before for Sir Henry Stanley and his followers who traced the course of the Congo, as the 2,718-mile long river was then called. This time, as Major J. Blashford-Snell reported, there were no hostile war canoes to face, and the inflatable boats used by the expedition were more serviceable than Stanley's Lady Alice, which had to be carried overland whenever rapids were approached. Stanley had no scientists in his party, but this time there were 50, with varied medical, anthropological, geological, zoological and botanical interests. The two latter groups were represented at the meeting by K. A. Joysey (University of Cambridge) and H. W. Woolhouse (University of Leeds), respectively.

Joysey and his colleague, A. Friday, were concerned particularly to look for specimens of the West African otter shrew (*Potamogale velox*), a large and vicious insectivore that lives in muddy water and is apparently active only at night. After a rendezvous with local pigmies, an otter shrew was eventually found, but proved elusive. The natives later presented the specimen, freshly killed, to the two zoologists who quickly removed the blood needed for serotaxonomic studies. The body is now in the Museum of Zoology at Cambridge.

Other aquatic animals proved less elusive, with local markets yielding a good catch of lung fish (*Protopterus*), elephant fish (various genera of the family Mormyridae) and paddle fish (*Polypterus*) needed for protein sequence studies of muscle aldolase. Throughout the four months of the expedition a party was also collecting fish—from the lakes, tributaries and headwaters—for the British Museum (Natural History). Other ichthyologically inclined members of the expedition collected parasites, bile salts (for an evolutionary study) or fossil specimens. But the entomologists acquired the largest collection. They took home 4,000 Lepidoptera, constituting 800 species, of which a proportion is considered to be undescribed. Experts at the British Museum (Natural History) are now working on these specimens, some of which will eventually be returned to the Government of Zaire. In the tropical rainforest the canopy level turned out to contain the greatest diversity of insect life, which seems to be quite distinct from the insect flora on the ground.

Throughout their journey, the three botanists on the expedition collected seeds of woody legumes for biochemical analysis of unusual amino acids. In southern Zaire, plant and soil specimens were collected for a study of the distribution of plants in relation to copper outcrops and the zonation of the flora was found to reflect the changing concentration of copper in the soil. A few species were growing in unusually toxic concentrations, with some lichens surviving on 5% copper. The material collected will be used in the search for the physiological basis of copper tolerance.

Data for a study of growth and productivity of *Papyrus*, with the aim of investigating the feasibility of making use of *Papyrus* again, was collected from the Upemba swamp basin, where the British botanists found working conditions none too pleasant. Another botanical project was a study of the frequency and distribution of plants which have the C4 pathway of photosynthesis. The outcome of this study will be reported later.

Nature, in the press; Herzenberg and Cantor, unpublished; Cantor and Pierce, unpublished) suppressor cells have an Ly phenotype (Ly 2,3) different from that of helper cells (Ly 1). They also possibly carry histamine receptors (Eichmann *et al.*, *Eur. J. Immun.*, **5**, 511; 1975) and may be Ia positive (Hammerling and Black, *Tenth Leucocyte Culture conference*, 1975). (See Table 1.) The view that at least some T suppressor cells may exert their effect by interaction with T helper cells rather than directly with B cells (Herzenberg and Cantor, unpublished) is supported by much previous data. This may well be a manifestation of helper and suppressor cells belonging to different subsets of T cells having complementary cell surface structures for interaction.

Recent work on idiotypes has shown that an antigen-specific receptor on T cells involved in help for antibody production and on T cells involved in anti-MHC (major histocompatibility complex) responses shares determinants with the immunoglobulin receptor for that antigen on B cells (Binz and Wigzell, *J. exp. Med.*, **142**, 197; 1975; Eichmann and Rajewsky, *Eur. J. Immun.*, in the press). If this receptor is not cytophilically acquired, this work has important implications concerning the nature of T cell receptors and suggests at least shared expression of a V region gene on both T and B cells, perhaps in association with an H region product (for example Taussig and Munro, *Nature*, **251**, 63; 1974). The fact that the idiotypes of receptors are antigenic and can provoke immune responses (refs above, and Jerne, *Harvey Lecture*, 1975, in the press, Lee *et al.*, *Nature*, **247**, 55; 1974) also opens up the possibility that an immune response to one antigen, once initiated, can provoke another immune response which limits or enhances the first response. This anti-idiotype response may, in turn, give rise to an anti-anti-idiotype response, and so on. This network theory has found favour amongst theorists and the data now accumulating suggest that anti-idiotype responses can occur during an immune response — but there is no good evidence as yet for their having a normal regulatory role.

The MHC plays an important part in cell–cell interactions during the induction of a whole range of cellular and humoral immune responses. In mice the MHC (H-2) is a series of loci mapping in the IXth linkage group (chromosome 17). Several other possibly analogous loci also exist on this chromosome. Of these the T/t region near the centromere has long fascinated developmental biologists because it governs a sequence of distinct steps in embryogenesis. These events are precisely defined by the recessive-lethal *T/t* alleles because each of them halts development at the very point where the functional allele would normally act. The prevailing supposition that morphogenetic processes of this kind must involve genetically programmed sensory devices of the sort implied by terms like "cell–cell recognition" is substantiated by the finding that the T/t genetic system, and at least one functional allele (+^{t12}) determines a distinct antigen (F9) that is expressed selectively on cells in a particular phase of differentiation. There seems to be a reciprocal relationship in the expression of this F9 antigen and the H-2 antigens—F9 in the embryo and H-2 in the adult. The *H-2* region lies close to *T/t* and is frequently tied to it in wild mouse populations by the presumably advantageous suppression of crossing over which is a property of *t* alleles. It is therefore of interest that genes at four loci within a region of some 15 centimorgans on chromosome 17 — *T/t, H-2K, H-2D* and *Tla*—have been shown to specify membrane glycoproteins of similar structure including the incorporation in each of them of a small subunit which is probably β-2-microglobulin. This was the main argument advanced by Artzt and Bennet (*Nature,* **256,** 545; 1975) in support of the hypothesis that this family of four loci are descendants of an ancestral locus that acquired the property of cell surface perception as a condition of metazoan evolution. The immune system can therefore be considered as a special case of adaptive differentiation using as its cornerstones genes which may be reduplications of genes coding for receptors that are important in more primitive cell recognition.

We are grateful to the Wellcome Trust for a contribution to travelling expenses. ☐

Slugs and plants

from Robert M. May

MUCH information about the natural history of plants and animals may be assembled in an orderly framework by noting that the world tends to provide a continuum of environments, ranging from the unpredictable (where evolutionary pressures are for rapid population growth to exploit the transient good times), through to predictable and biologically crowded environments (where evolutionary pressures are to be a good competitor). These notions, which reach back to Darwin, are commonly formalised in terms of the extremes of "r-selection" and "K-selection", respectively (see *Nature,* **257,** 737; 1975).

One application of these ideas is to the phenomenon of succession This phenomenon, whereby untended open space tends to revert to weeds, then shrubs, and eventually to woodland, is familiar to all possessors of suburban lawns. The temporal procession from early succession to late succession and climax is a paradigmatic example of a gradient from r-selection to K-selection. On a small scale, such gradients from r- to K-selection have been documented in populations of dandelions (Gadgil and Solbrig, *Am. Nat.,* **106,** 14–31; 1972), goldenrods (Abrahamson and Gadgil, *Am. Nat.,* **107,** 651–661; 1973) and other wild flowers growing in patches subject to significantly different degrees of disturbance McNaughton (*Am. Nat.,* **109,** 251–261; 1975) has recently supplemented this work with a meticulous study of populations of cattails (*Typha*) in a variety of sites with differing lengths of frost-free growing periods. He shows the relatively unpredictable locations with a short growing season favour r-selected genotypes which have faster development, produce more offspring (seeds), but invest less energy in each offspring (smaller seeds) and put less premium on traits conferring competitive ability, such as height (to shade out other plants).

A somewhat deeper test of the ideas comes from considering the relation between plants and the beasts that eat them, as a function of successional stage. Compared with late successional plants, those in early succession should invest relatively more of their resources in growth (and reproduction), and relatively less in defence against herbivores. This is so, both for the reasons outlined above, and because the patchy and unpredictable distribution associated with early succession itself provides a degree of escape from herbivores. Thus early successional plant species should provide better food sources for generalised herbivores than do later successional and climax plants. Cates and Orians (*Ecology,* **56,** 410–418; 1975) have recently tested this by taking 100 plant species, of three growth forms and from different seral stages, and determining their short-term palatability to two slug species, one native to western Washington (*Ariolimax columbianus*) and one introduced from Europe (*Arion ater*). Palatability of a given test plant was measured as the ratio of test material eaten to control material eaten, in a well-defined laboratory setting. The results showed that early successional annuals were significantly more palatable than early successional perennials, which in turn were significantly more palatable than later successional species. This validation of theoretical expectations was clear cut, in that no correlation was found between palatability and evolutionary association of the herbivores with particular plant species.

Specifically, Cates and Orians define a "palatability index" as the ratio of log (amount of test material eaten) to log (amount of control material eaten). Then for the native slug *Ariolimax,* the average palatability index for early successional annuals and biennials was 0.96, for early successional perennials 0 77, and for later successional and climax plants 0.46. The corresponding figures for the European slug *Arion* were 0.99, 0.69, 0.40.

A more detailed field and laboratory study of one particular slug–plant system has also been carried out by Cates (*Ecology,* **56,** 391–400; 1975). Using wild ginger (*Asarum caudatum*) and again the native slug *A. columbianus,* he showed the plant populations were polymorphic for growth rate, seed production, and palatability to the slug. In habitats where slugs were relatively rare, populations of wild ginger were dominated by individuals allocating more energy to growth rate and seed production, and less to the production of biochemicals that slugs find nasty. Conversely, in habitats where the slugs were abundant, the more K-selected individuals predominated.

Cates and Orians note that slugs are not "representative" generalised herbivores, because they tend to avoid grasses. Nonetheless, the successional trends they have documented march with the results of other studies, and indicate "broad patterns in the ways plants and herbivores have mutually influenced one another's evolution."

Biochemical factors in schizophrenia

from Richard Rodnight

THE paper by A. G. M. Pearson and A. J. Turner in this issue of *Nature* (page 173) bridges two areas of research—transmethylation and folic acid metabolism—both of which have potential significance for studies of the biochemical basis of psychotic behaviour in man. A disturbance of transmethylation has often been proposed as a possible factor in the aetiology of schizophrenia, but until recently there were few clues as to the pathways which might be involved. A possible mechanism came to light with the discovery in 1961 by J Axelrod (*Science,* **134,** 343) of a methyltransferase enzyme in rabbit lung that transfers methyl groups from S-adenosylmethionine (SAM) to the naturally occurring indoleamine tryptamine to form mono- and dimethyltryptamine (DMT). The latter compound has been known for many years to induce in normal subjects a model psychosis when administered parenterally, an observation which evokes the suggestion that some of the typical symptoms of schizophrenia may be due to the aberrant production of endogenous DMT in the affected individuals. There is indeed good evidence now that the body does convert minute fractions of its stores of

The bee language controversy

from John R. Krebs

Karl von Frisch's Nobel Prize winning discovery of the dance language of honeybees is one of the classics of biology. Over many years von Frisch collected evidence to show that when a successful foraging bee returns to the hive it is able to recruit other workers by communicating the precise distance and direction to the food source, encoding this information in the abstract symbolism of a 'dance' performed on the hive surface. The distance is encoded in the speed of dancing and the direction by the orientation of the figure-of-eight dance in relation to gravity. This remarkable feat places honeybees second only to man and perhaps chimpanzees in the use of abstract symbols in transmission of information.

About eight years ago, Adrian Wenner pointed out that two of von Frisch's best known experiments were not as convincing a demonstration of the dance language as most people had thought. In these two studies, known as the 'step' and 'fan' experiments, von Frisch showed that naive recruit bees would go specifically to experimental food dishes indicated by the dances of experienced foragers, and ignore identical nearby control dishes. In the fan experiment the control and experimental dishes were at the same distance from the hive but in different directions, and in the step experiment the dishes were in the same direction but at different distances. Wenner correctly pointed out that not only is the experimental dish in these experiments unique in being the one indicated by the dance, but also it is the one towards which experienced foragers are flying. The recruits could either be using the dance information or simply watching where the foragers go. Wenner eliminated this confounding variable by training equal numbers of foragers from a separate (control) hive to go to each of the control dishes, and he now found that recruits from the experimental hive no longer preferred the dish indicated by the dance. Wenner's conclusion from this and other experiments was that recruits may be stimulated to leave the hive by the dance, but that they normally search for food by smell, using the smell of the dancing foragers as a guide.

Naturally enough, Wenner's results aroused enthusiastic criticism, followed by new experiments supporting von Frisch's story, with further counterclaims by Wenner and his colleagues, culminating in a headline in *Nature* (**241**, 171; 1973) suggesting that von Frisch's data could be explained without recourse to the dance language theory. One valuable consequence of Wenner's criticisms was that people devised more rigorous tests of bee dance communication, and the latest of these studies, by J. L. Gould (*Science*, **189**, 685; 1975) finally shows beyond doubt that bees use the dance information.

In a most beautifully ingenious experiment, Gould exposed recruit bees to conflicting information: the dance indicated one direction, while the foragers that gave the dance flew in another direction. The key to Gould's experiment is the fact that bees can be persuaded to orientate their dance in relation to the Sun or (in the case of Gould's experiment) a bright light instead of gravity. As long as the recruits can also see the Sun or bright light, they automatically 'read' the dance correctly, using the light source instead of gravity as a reference. What Gould did then was to cover the ocelli of the dancing foragers with black paint so that they could not see the artificial Sun (a 650 watt bulb) shining into the hive but could still forage and dance. The returning foragers now oriented their dance with respect to gravity, as usual in a dark hive, but the recruits interpreted the dance as though it were oriented with respect to the bright light. To give an example, suppose the bright light is placed at 60° to the vertical, and a returning forager has just come from a food dish directly in line with the real Sun outside. The forager dances on the vertical surface of the hive, unaware of the bright light, and orientates her dance vertically upwards (which is the gravity-based code for 'towards the Sun') The recruits, however, read the information as '60° from the line of the Sun', and head off in a different direction from the foragers. Recruits always arrived at food dishes in the direction indicated by their interpretation of the dance, and not at the experimental dish used by the foragers. Further, by moving the bright light to different positions in the hive, Gould could get the recruits to go to different feeding dishes—always the one predicted by incorrect reading of the dance

It is also particularly satisfying that Gould was able to show at least one reason why Wenner did not find any effect of the dance on recruits. In the initial part of any dance experiment, a few chosen foragers are trained to fly to a particular dish, this being the dish they indicate to naive recruits in the dance. Wenner used training dishes scented with the same odour as was later used in the experiment, while Gould was careful to change to a new scent on the day of the experiment. If Gould used the same scent for both training and experiment, he got the same results as Wenner: the recruits went to any dish with the same scent as that carried into the hive by foragers. The conflict between the von Frisch and Wenner results is at least partly due to details of experimental design and, as Gould points out, this might well mean that recruit bees use the dance information when they are just starting to exploit a new food source but use scent, as Wenner proposed, when exploiting the same food source at a later stage.

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indoleamines to N-methylated products with hallucinogenic properties. Traces of both the mono- and dimethylated derivatives of tryptamine and serotonin are excreted in human urine and may occasionally be found in blood; the excretion, moreover, does not seem to be derived from the diet or bacterial action in the bowel. There are also reports, as yet unconfirmed and inadequately documented, that mentally ill patients with the diagnosis of non-affective psychosis tend to excrete more DMT in their urine than do mentally ill patients with other diagnoses or normal subjects. It is, however, uncertain how much, if any, of urinary DMT is derived from tryptamine methylated in the brain. There is no doubt that human lung contains an SAM-dependent methyltransferase which forms DMT from tryptamine, the enzymic basis for the same reaction in mammalian brain is now much less clear than formerly suspected. Earlier reports on the occurrence of an indoleamine methyltransferase in brain using SAM as methyl donor were based on unsatisfactory methods of product identification and the reaction urgently needs reassessing by more precise methods such as gas chromatography and mass spectrometry.

Suspicions that folic acid might be involved in the endogenous formation of hallucinogens in the body arose about 3 years ago when several reports appeared indicating that besides SAM,

N^5-methyltetrahydrofolic acid (5-MTHF) could act as methyl donor for the N-methylation of biogenic amines in the brain; dopamine, apparently yielding its N-methyl derivative epinine, appeared a particularly good substrate but it was also claimed that both tryptamine and serotonin could be N-methylated in this system. Unfortunately product identification in these experiments was no better than in the parallel studies using SAM as donor. The usual procedure has been to incubate a crude mixture of soluble proteins from the tissue under study with radioactive SAM or 5-MTHF in the presence of acceptor substrate and then to extract the labelled products with solvents and fractionate them on one or more TLC systems, comparing migration rates with those of authentic compounds. When the combined method of gas chromatography/mass spectrometry was applied by several workers to the products produced in the 5-MTHF-dependent reaction it became evident that methylation of the side-chain amino groups in the case of either dopamine or the indoleamines does not occur. With dopamine as acceptor the product was shown to be a tetra-hydroisoquinoline alkaloid (Laduron and Leysen, *Biochem. Pharmac.*, **24**, 929; 1975) and with the indoleamines formation of the corresponding tetrahydro-β-carbolines was demonstrated (Wyatt *et al. Science*, **187**, 853; 1975). Further examination of the nature of the reaction showed that it consists essentially of the enzymic formation of a 1-carbon compound from 5-MTHF followed by nonenzymic condensation with amine and cyclisation to give the products mentioned above The 1-carbon unit was shown by Laduron to be formaldehyde, a compound which would be expected to react with aromatic alkylamines by the Pictet-Spengler condensation to give the corresponding cyclic compounds. What then is the mechanism by which formaldehyde is formed enzymically from 5-MTHF? The answer to this question has been given simultaneously by Pearson and Turner (this issue) and R. T. Taylor and M. L. Hanna (*Life Sci.*, **17**, 111–120; 1975). Both groups of workers show that formaldehyde production from 5-MTHF is a result of the activity of the widely distributed enzyme methylenetetrahydrofolate reductase, which is present in crude tissue extracts. This enzyme normally catalyses the NADPH-dependent formation of 5-MTHF from N^5, N^{10}-methylene tetrahydrofolate; in suitable conditions and in the presence of electron acceptors it may act in the reverse direction to yield from 5-MTHF, methylene tetrahydrofolate, which in turn is known readily to disocciate into tetrahydrofolate and formaldehyde. Pearson and Turner have purified the reductase from pig liver by gel filtration and isoelectric focusing and in the process were unable to separate it from the putative dopamine methyltransferase activity. Taylor and Hanna investigated the previously reported stimulation of the enzyme activity by FAD and methylcobalamin (an observation earlier deduced as evidence for an involvement of N^5-MTHF homocysteine methyltransferase in the N-methylation of biogenic amines) and concluded it is a result of the ability of these compounds to act as nonspecific electron acceptors

While these observations tend to discount a role for folic acid in the endogenous formation of N-methylated hallucinogens it is of interest to note that the β-carboline products bear a close structural resemblance to the alkaloids harmine and harmaline, compounds which reputedly possess psychotomimetic properties in man. Moreover a case of "folate-responsive schizophrenia" in an adolescent girl has been described (Freeman *et al.*, *New Engl. J. Med.*, **292**, 491, 1975). This patient presented with many of the typical symptoms of schizophrenia and was found on investigation to have a genetically determined deficiency in the activity of the enzyme 5, 10-methylenetetrahydrofolate reductase. The enzyme defect resulted in a decreased formation of 5-MTHF and in homocystinuria due to inefficient remethylation of homocysteine to methionine The patient's symptoms dramatically improved on administering high doses of folic acid, but reappeared when the therapy was discontinued. It looks as though the complex interrelations of the cerebral metabolism of methionine, folic acid and the cobalamines may remain a fruitful area of research for those workers interested in the role of biological factors in the genesis of the schizophrenic syndrome. ☐

Angular momenta of planets and asteroids

from David W. Hughes

REGULARITIES in the contemporary structure of the Solar System provide clues not only to the primordial conditions in the system, but also to the accretion mechanisms that formed the planets and satellites and to the subsequent collisions that have taken place between these bodies. The Titius-Bode laws (relating planetary and satellite spacings to their place in the numerological sequence) and the fact that the majority of planets and satellites move in prograde, near circular orbits which lie close to the ecliptic plane are two regularities that have been known for a long time. It is also well known that most planets and satellites spin about axes which are perpendicular to the ecliptic. The relationship between the rotational angular momenta of planetary bodies and their masses is a more recent discovery, however, being first noticed by MacDonald (*Space Sci. Rev.*, **2**, 473; 1963).

The angular momentum of a body is simply the product of its angular spin velocity, ω, and moment of inertia, I. (The moment of inertia is given by $I=Mr^2\alpha$ where M is the mass, r the radius and α the gyration constant; α is 0.4 for a solid equidense sphere). Figure 1 is a logarithmic plot of the angular momenta of planets and asteroids as a function of their masses, the line in Fig. 1 having the form

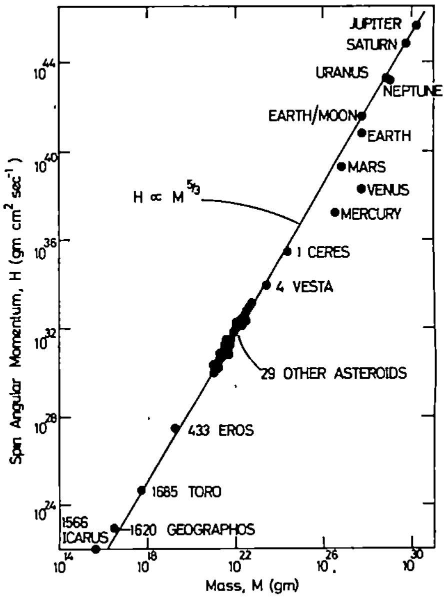


Fig. 1 A logarithmic plot of the spin angular momenta of planets (with the exception of Pluto) and 35 asteroids with well-determined sizes, shapes and rotation rates as a function of their masses (after Fish, *Icarus*, **7**, 251; 1967, Hartmann and Larson, *Icarus*, **7**, 257; 1967 and Burns, *Icarus*, **25**, 545; 1975).

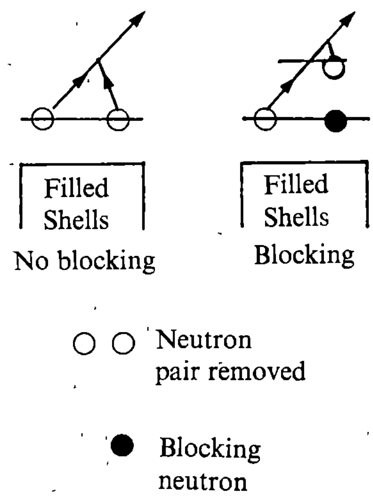
$H=1.47\times10^{-5}M^{5/3}$ where H and M are both in c.g.s. units T, the rotational period, is found by substituting $T=2\pi/\omega$ into the above equation. This

Neutron blocking in alpha-transfer

from P. E. Hodgson

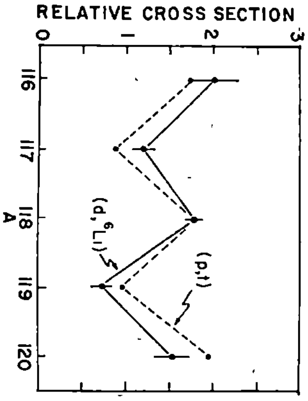
NUCLEAR reactions often give us very direct information about the detailed shell structure of nuclei. This is shown particularly well by the many studies of single-particle states by one-nucleon transfer reactions. In these reactions, a nucleon is either removed from a nucleus or given to it, and study of the angular distribution of the outgoing particles tells us the quantum numbers and the occupation probabilities of the single-particle state involved (*Nature*, **245**, 74; 1973, **249**, 695; 1974).

There are also some reactions in which more than one nucleon is transferred at the same time, and these can sometimes tell us about the nucleons that are not participating directly in the reaction. Such nucleons can get in the way, or 'block', the reaction, thus reducing the probability of its taking place



The first examples of this were found in studies of the (p,t) reaction, which removes two neutrons from the nucleus. In a nucleus with an even number of nucleons both neutrons can be removed from the same single-particle state, whereas if there is an odd number of neutrons it may be necessary to take the neutrons from different states. This latter process is more difficult, and the cross section for the reaction is reduced by a factor of about two. In this way a neutron that does not participate directly in the reaction nevertheless makes its presence felt and thus gives us information about nuclear structure (Fleming, Blann and Fulbright, *Nucl. Phys.*, **A163**, 401; 1971).

This blocking effect has now been observed in alpha-transfer reactions as well. Becchetti and Jänecke (*Phys. Rev. Lett.*, **35**, 268; 1975) have measured the cross sections of several (d,^{6}Li) reactions on even and odd isotopes of tin. The reactions on the even isotopes can just remove the two neutrons from one single particle state and the two protons from another, but the reactions on the odd isotopes have to take the neutrons from different states. This is more difficult, and as shown in the figure the corresponding cross sections are again reduced by a factor of about two.



Comparison between the relative cross sections of (p,t) and (d,^{6}Li) reactions as a function of A for some tin isotopes.

This blocking in alpha-transfer is very similar to the same effect in (p,t) reactions, and suggests that the mechanism is essentially the same, with the two transferred protons acting simply as spectators. The regularity of the reduction suggests that it is the $3S_{1/2}$ state that has an odd nucleon in the odd isotopes, and this is expected from the simple shell model.

The spectroscopic factors obtained by analysing the data with the distorted wave theory show a similar reduction for the odd isotopes, confirming that it is not simply due to the differences in binding energies and Q-values, but is a real nuclear structure effect.

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gives $T=4.3\times10^5\alpha r^2M^{-\frac{1}{2}}$. If the body is assumed to have a constant density, ρ, throughout (that is, $M=1.33\ \pi r^3\rho$) and an α of 0.4 then $T=6.59\times10^4\rho^{-\frac{1}{2}}$, which, when the assumption is made that all Solar System bodies have the same density and no central mass concentration, confirms Alfvén's findings (*Icarus*, **3**, 52; 1964) that all planets and asteroids have approximately the same period of rotation. Using a density of 3 g cm^{-3} this period comes to 8.8 h.

It can be seen from Fig. 1 that Mercury and Venus have considerably lower angular momenta than predicted by the linear relationship. This is due to tidal interactions between these planets and the Sun. The high rate of spin deceleration suffered by Venus indicates that the solid dissipation inside the planet is considerable. This is only possible if large regions in the interior of Venus are near their melting point thus enabling creep, elastoviscosity and 'direct' viscous interactions to play their part in dissipating energy. Combining the angular momentum of the spinning Earth and the orbital angular momentum of the Moon gives a point on the line in Fig. 1 indicating that the angular momentum of the Earth–Moon system has not changed much during the history of the Solar System. All that has happened is that the Moon has moved further away, and the Earth now spins more slowly.

In a recent paper in *Icarus* (**25**, 545; 1975) Burns doubles the number of asteroid points used in Fig. 1. Accurate sizes based on the radiometry and polarimetry work of Chapman, Morrison and Zellner (*Icarus*, **25**, 104; 1975) are used together with an attempt to include for the first time the effect of an asteroid's non-sphericity. The brightness of asteroids vary with periods scattered around 9 h. These brightness variations, which in some instances range up to two magnitudes but are more typically 0.2 magnitude, are primarily caused by the asteroid having an irregular shape and have been used by Burns to deduce their shape. As the asteroids usually rotate about their principal axes of maximum moment of inertia they contain more angular momentum per unit mass than a spherical body. The effect of inter-asteroid collisions should make the smallest asteroids spin faster and be more irregular. It can be seen in Fig. 1 that 1566 Icarus and 1620 Geographos both lie above the straight line, indicating that they are spinning faster than expected.

Why is the spin period around 9 h? It seems that the accretion processes which formed the planets and asteroids become less and less efficient as these bodies get nearer and nearer to their rotational stability limit. This limit occurs when the rotational surface velocity of the body is equal to its escape velocity (that is when $T\approx3.3\rho^{-\frac{1}{2}}$ h, a rotational period of ~ 2 h for a body of density 3 g cm^{-3}). A conclusion might be that bodies cannot accrete if they spin faster than about one third the rotational stability limit.

Deviations of the spin axes from the normal to the ecliptic are probably due to the statistical fluctuations in the final accumulation of any planet, in other words Uranus' unusual 98° obliquity could be produced by the angular momenta brought in by the last few accreting planetismals. This statistical fluctuation in the accretion process also causes the scatter in the asteroid data.

Two other questions are of interest. First, do these collision-induced spin rates in asteroids ever become so rapid that the surface layers are not gravitationally bound? Excessive spin rates could sweep away any regolith and leave a clean surface. Burns concludes that this does not happen. Even in the case of 1566 Icarus, the smallest and fastest spinning asteroid of known rotation and shape, and 321 Florentina the second most rapid rotator, their densities have to be less than 2.8 and 2.3 g cm^{-3} respectively for this to occur. As asteroids seem to fall into two main compositional groups, however, (Chapman *et al.*, *Icarus*, **25**, 104; 1975) similar to carbonaceous chondrite meteorite (Type I has $\rho=2.5$ g cm^{-3}, Type II has $\rho=2.9$ g cm^{-3}) and to stony-iron meteorites ($\rho=5.0$ g cm^{-3}) things are getting pretty close.

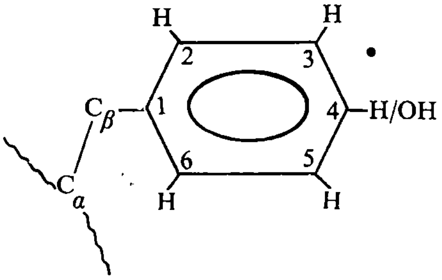
Second, are the spin rates fast enough to cause internal tensile stresses which over long periods of time, split the asteroids apart. Burns again concludes that the answer is no and that if forces are attractive on the surface of the asteroid they are also attractive throughout the interior.

In the past asteroids might have been spinning about other axes than that of maximum moment of inertia. The spin rate would then be faster and these excess rates could have peeled off the requisite outer layers to produce a stable configuration. The fact that carbonaceous chondrite material is very friable and that 1566 Icarus and 321 Florentina are surprisingly spherical for their sizes and rotation rates adds credence to this idea; however more observations of small asteroids are needed to confirm this thesis.

Flipping rings in proteins

from G. C. K. Roberts

X-RAY crystallography has given us a very detailed picture of the structure of many proteins. This picture is, however, a static one, and in recent years there has been much interest in attempts to understand in detail the dynamics of protein structure. Nuclear magnetic resonance (NMR) spectroscopy is potentially able to give information on the rate of motion of individual amino-acid sidechains within a protein. The complexity of the NMR spectra of proteins often makes this information difficult to extract, but recently several reports have appeared which focus on the motional characteristics of the aromatic rings of tyrosine and phenylalanine residues.



Sykes and his colleagues have been studying the alkaline phosphatase of *Escherichia coli*, labelled *in vivo* with 3-fluorotyrosine. The ^{19}F NMR spectrum shows a separate resonance for each of the 11 tyrosine residues per subunit, and a thorough study of the ^{19}F relaxation behaviour has allowed Hull and Sykes (*J. molec. Biol.*, **98**, 121, 1975) to draw a number of interesting conclusions about the motion of these tyrosine side chains. They observed that the linewidth of the ^{19}F resonances (related to the relaxation time T_2) was markedly frequency dependent, becoming greater at higher frequency. One relaxation mechanism which would be expected to give rise to a frequency-dependent T_2 is chemical shift anisotropy relaxation. The chemical shift of a nucleus is a tensor quantity which depends on the orientation of the molecule with respect to the external magnetic field. In liquids, the tumbling of the molecule averages the chemical shift over all orientations, but the resulting fluctuations in Zeeman energy provide a relaxation mechanism which is most effective at high fields and for nuclei (such as ^{19}F) which have a large chemical shift anisotropy. The linewidths of 9 of the 11 fluorotyrosine resonances could be explained in this way, and this analysis led to estimates of the rate of internal motion about the C_β–C(1) bond ranging from 10^6 to 10^8 s^{-1} (the overall tumbling of the protein having a rate of about 2×10^6 s^{-1}). For two of the tyrosines, there was an additional contribution to the linewidth from exchange, and for these the rate of reorientation of the ring was slower, in the range 10^2–5×10^4 s^{-1}. Analysis of the spin-lattice relaxation time and of the dipolar contribution to T_2 led to an upper limit for motion about the C_α–C_β bond of 10^6 s^{-1}.

In favourable circumstances, analysis of the ^{1}H spectrum of tyrosines and phenylalanines in proteins can give a rather direct indication of the rate of rotation about the C_β–C(1) bond. This bond lies along the symmetry axis of the aromatic ring (see figure). In the free amino acids or simple peptides, the 2 and 6 protons and the 3 and 5 protons are equivalent, and a simple spectrum is observed. For a tyrosine or a phenylalanine in a protein, such a simple spectrum will be observed only if either the environment of the two sides of the ring is identical—an unlikely coincidence—or the ring is rotating sufficiently rapidly for the 2 and 6 and 3 and 5 protons, respectively, to experience an average environment. Roughly speaking, a rotation about C_β–C(1) of approximately 200 s^{-1} will be sufficient to produce this averaging.

In hen lysozyme, all three tyrosine residues show simple spectra indicative of averaging about C_β–C(1) (Campbell, Dobson and Williams, *Proc. R. Soc. Lond.*, **B189**, 503–509; 1975). The bovine basic pancreatic trypsin inhibitor has four tyrosine residues, whose resonances have been assigned to individual residues in the sequence (Snyder *et al.*, *Biochemistry*, **14**, 3765; 1975). Three of these show simple spectra at room temperature, while the fourth, Tyr 35, shows a complex spectrum indicative of slow rotation (Snyder *et al.*, *op. cit.*, Wüthrich, presented at *Conference on Molecular Structural Methods in Biological Research*, *Stanford*, Oct. 9-10, 1975). Similar phenomena are seen for the phenylalanine residues (Wüthrich and Wagner, *FEBS Lett.*, **50**, 265; 1975). Values for the activation energies can be obtained by temperature studies (Wüthrich, *op. cit.*).

It thus seems to be rather common for the aromatic rings of tyrosine and phenylalanine residues in proteins to rotate (the motion is in all probability essentially a flip through 180°) at rates greater than 200 s^{-1}. Some of the implications of this are brought out in a recent paper by Gelin and Karplus (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2002–2006; 1975). These authors have calculated the energy barriers to rotation about C_β–C(1) of the tyrosines of the basic trypsin inhibitor, based on its crystal structure, which is known to high resolution. For both the crystallographically determined structure and an energy-refined version thereof, the barriers are extremely high (up to 230 kcalorie mol^{-1}). If the protein structure was allowed to 'relax', however, (simulated by energy minimisation with the tyrosine ring in its minimum and maximum energy orientations), a drastic reduction in the barriers could be achieved at the cost of a rather modest amount of bond angle strain. In this case, at least, it appears that a significant "breathing" of the protein is required to permit the aromatic rings to flip at the rates implied by the NMR experiments.

review article

The protein gap

J. C. Waterlow & P. R. Payne*

The concept of a worldwide protein gap, derived from the diagnosis of kwashiorkor as a protein deficiency state, is no longer tenable. Current estimates of children's protein and energy requirements are considered realistic, and by these criteria the problem is mainly one of quantity rather than quality of food.

"It is a most nourishing and stimulating diet to eat one's own words" (attributed to Sir Winston Churchill)

In 1968 the UN Advisory Committee on the Application of Science and Technology to Development presented a report to UNESCO with the title "International action to avert the impending protein crisis"[1]. Numerous recommendations were made about methods of increasing protein supplies, the production of high protein foods and the exploitation of unconventional sources of protein to fill the "protein gap". Seven years later there is a strong body of opinion that this is an incorrect statement of the problem: that what the world with its expanding population has to face is primarily a food gap or an energy gap and not a protein gap. McLaren, in his stimulating article "The great protein fiasco"[2], has summarised the history of this preoccupation with protein and in particular the reasoning by which the UN agencies came to identify protein as the weak point in the world's nutritional defences. It all began with kwashiorkor.

When Williams described kwashiorkor in young children in Ghana some 40 years ago[3], she diagnosed it as a nutritional disease, adding cautiously "... in which some amino acid or protein deficiency cannot be excluded". The children who developed the disease did so after weaning, when they were fed on starchy porridges rather low in protein, and they were cured by milk. It took some time for the diagnosis of protein deficiency to be generally accepted; in earlier accounts, particularly from Latin America, the presence of oedema and skin lesions in many cases led to the suggestion of a multiple vitamin deficiency. For the past 25 yr, however, the view has been fairly generally held that the two extremes in the spectrum of childhood malnutrition—kwashiorkor and marasmus—represent conditions in which the main limiting factor is protein on the one hand, energy on the other. This satisfying hypothesis is epitomised in the title of a book published in 1968—*Calorie Deficiencies and Protein Deficiencies*[4].

The chain of argument which leads from these early clinical studies to the concept of the protein gap as a worldwide problem rests on three premises: that kwashiorkor is indeed a manifestation of protein deficiency; that it is very common in the developing world; and that wherever kwashiorkor occurs milder forms of protein deficiency must be widespread, affecting far more children than those who develop the severe disease. This is the "tip of the iceberg" theory. All three premises need to be examined.

Is kwashiorkor a result of protein deficiency?

The evidence is to a large extent circumstantial. Retrospective dietary histories are of limited value because the illness itself causes a fall in appetite and thus a reduced intake of all nutrients. The most powerful evidence of cause and effect in nutritional work—the therapeutic test—is not practicable, because a sick child cannot be fed protein alone. Brock *et al.*[5] attempted this approach when they produced "initiation of cure" (loss of oedema) with a diet in which the only source of nitrogen was a mixture of pure amino acids, but they had to give a source of energy as well. Some of the biochemical features of kwashiorkor, notably the low serum albumin and altered amino acid pattern[6,7], indeed suggest a state of protein depletion. Nevertheless, our understanding of the pathogenesis of the major features of kwashiorkor, oedema and fatty liver, is still incomplete. It is reasonable to suppose that oedema results in part from the low serum albumin concentration, although not all workers agree. Evidence suggests that the most likely cause of fatty liver is a failure of synthesis of the protein part of the lipoprotein which is responsible for fat transport out of the liver[8,9]. Animal experiments have also provided useful evidence: the rat is a poor model, but many of the features of kwashiorkor have been produced in pigs[10] and in monkeys[11] by feeding a diet high in carbohydrate and low in protein. Grimble and Whitehead[12] found, however, that in pigs the biochemical changes typical of kwashiorkor did not appear until the appetite of the animals had fallen, so that their food intake was reduced. The evidence is therefore not clear cut, but one difference between kwashiorkor and marasmus is undeniable: children with kwashiorkor must have been less energy-deficient than those with marasmus, for the simple reason that their bodies contain more fat, as is immediately obvious at autopsy.

The first serious attack on the conventional theory came from Gopalan in India[13]. In a prospective study of children in a poor community no quantitative or qualitative differences in the diet were found between children who developed kwashiorkor and those who became marasmic. Gopalan therefore suggested that it is not the diet which determines the clinical picture, but the way in which the subject adapts or fails to adapt. One explanation[14] (which does not take us very far) for a difference between children in their response to an inadequate diet lies in the known variability between individuals in their requirements for energy and for protein. One could conceive that on a diet which is marginally deficient in both, a child who happened to have a high energy requirement would develop marasmus, and one with a high protein requirement would develop kwashiorkor.

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The basis for intra-individual variation in requirements is quite unknown. One may speculate a little further. When energy intake is inadequate, protein, mainly from muscle, is oxidised to fill the gap. It has been said that the marasmic lives on his own meat; that is, that the net efflux of amino acids from muscle provides enough substrate for synthesis by the liver of albumin and apolipoprotein, so that the characteristic features of kwashiorkor do not appear. The release of amino acids from muscle, and the extent to which they are utilised for protein synthesis in other tissues or for gluconeogenesis, probably depends on the balance of at least three and possibly four hormones—insulin, cortisol, glucagon and growth hormone. Whitehead *et al.*[15] have described progressive disturbances in endocrine balance in kwashiorkor and there is some evidence of endocrine differences between kwashiorkor and marasmus[16]. Again we do not know whether these are effects of distinct causes or whether they represent the inherent variability of response.

Finally, there are differences, which may be important, in the natural history of kwashiorkor and marasmus. It is often said that marasmus typically occurs at an earlier age, but this is not universally true. Within each of the three countries—Jamaica, Iraq and Jordan—for which we have comparable data, there seems to be no significant difference in the age at which the different types of case present at hospital. In all three series, however, the marasmics were more stunted in height than the children with kwashiorkor[17-19]. The same has been found in unpublished studies from Sudan and Nigeria. This is clear evidence that marasmus is a more long standing condition and that its onset must have been earlier. It has also been suggested that kwashiorkor is typically precipitated by an acute infection such as measles, whereas marasmus infection for example, gastroenteritis[2], tends to be chronic and recurrent.

Table 1 Estimates of protein and energy requirements at 1 yr of age

Year	per kg body weight Protein* (g)	Energy† (kcal)	 (kJ)	Protein energy / Total energy (%)	Source	Reference
1948	3.3	100	420	13.2	NRC (USA)	47
1957	2.0	100	420	8.0	FAO	48
1964	2.5	100	420	10.0	NRC (USA)	47
1965	1.1	100	420	4.4	FAO/WHO	49
1968	1.8	100	420	7.2	NRC (USA)	47
1969	1.3	110	460	4.7	DHSS (UK)	50
1973	1.27	105	440	4.8	FAO/WHO	27
1974	1.35	100	420	5.4	NRC (USA)	47

*As protein which is 100% utilised.

†Units of energy as in original publications Rounded off to 4.2 kJ = 1 kcalorie.

Many readers may regard this discussion of the characteristics of childhood malnutrition as academic and irrelevant to the question of the protein gap, but it is important to realise how much is still unknown about the way in which children adapt or fail to adapt to deficient diets. This lack of knowledge has practical importance.

Is kwashiorkor common?

When the WHO and FAO became concerned about childhood malnutrition after the war, one of the first results was a survey in Africa by Brock and Autret[20]. Their report concluded that "kwashiorkor is the most serious and widespread nutritional disorder known to medical and nutritional science". McLaren[2] has pointed out that this judgment, based largely on rural Africa, ignores large areas of the world where marasmus is by far the commonest form of malnutrition. It is also frequently said that kwashiorkor is a rural, marasmus an urban, disease; and that marasmus is getting commoner because of increasing urbanisation and decreasing breast feeding. These statements are plausible, but they are not supported by firm epidemiological evidence. The reason is not just a lack of adequate surveys, but also the lack of an agreed and standardised system of reporting, without which it is impossible to make comparisons between different regions or to identify changes that are occurring with time. If, as the literature suggests, the prevalence of kwashiorkor differs in different countries with different dietary patterns, there should here be an important clue to aetiology[17]. This clue has not, however, been systematically followed up.

Is there evidence of widespread protein deficiency in the absence of clinical disease?

Estimates by WHO[21,22] that in many countries 20–40% of children are moderately or severely malnourished are in most cases based on a deficit in weight for age. This growth failure is, of course, a nonspecific effect of malnutrition. It is possible that more information may be obtained by analysing separately two components of growth failure: inadequate gain in weight and inadequate gain in length or height[17,23].

The search for specific biochemical indicators of protein deficiency has been going on for many years. Whitehead, a pioneer in this field, has concluded that the most sensitive index of impending kwashiorkor is a decrease in serum albumin concentration[24]. The greater precision of modern automated methods makes it possible to detect small deviations from accepted normal levels that are statistically significant. It remains to be proved, however, that this change is indeed a specific effect of protein deficiency.

The third approach is by comparison of intakes with requirements. Before going on to this we have to consider the basis on which requirements have been estimated, since they are often considered not to be realistic, or little better than guesses.

Estimates of requirements

Table 1 shows how estimates of the requirements of a 1-yr-old child for protein and energy have changed over the years.

Protein requirements. The figure of 1.27 g kg^{-1} in terms of milk protein is in our view very well founded. It is easier to estimate the requirement of an infant than of an adult because growth provides an extremely sensitive criterion of whether the child's needs are being met. In infants up to about 6 months the requirement is based on the observed intakes of infants consuming breast milk freely and growing normally[25]. This could even be slightly more than the minimum requirement. At about 1 yr the requirement has been derived from measurements of the minimum amount of cow's milk protein necessary to secure normal growth[26]. As might be expected, it is slightly less per kg than that of the younger infant who is growing faster. Of the requirement of 1.27 g kg^{-1}, it is calculated that about seven-eighths is needed for maintenance and only about one-eighth for growth[25]. We have found that if the protein intake of infants is reduced from an adequate to a maintenance level, with no change in energy, growth falls off within 1 or 2 d. It seems that the amount used for maintenance is so closely fixed that there is no margin to supply the needs for growth if the intake is slightly reduced.

In an adult man the latest estimate of protein requirement[27], in terms of egg protein, is 0.57 g kg^{-1} d^{-1}: about half that of the 1-yr-old child. This figure is derived from the amounts of protein needed to secure nitrogen balance. For an adult of average weight this comes to about 40 g of milk or egg protein per d, or about 60 g if corrected for the lower protein quality of the average mixed diet. This is about two-thirds of the amount of protein eaten daily by most people in this country. Many people feel strongly that these estimates of protein requirement are unrealistically low, and that the protein gap has been artificially abolished by the stroke of a pen, simply by reducing the requirement. If the estimates for infants are well founded, however, it is difficult to see why those for adults should not be correct. The lower requirement for the adult is not due simply to the absence of growth; the adult is considered to have a lower maintenance requirement, and this makes sense, because the rate of many metabolic reactions decreases with increase in body weight. Examples are the basal oxygen uptake, the rate of total protein turnover[28] and the rate of albumin synthesis[29,30]. Young *et al.* have shown a close correspondence between protein requirements and protein turnover rate over a whole life span, from prematurity to old age[31].

Table 2 Protein quality of cereals determined in different ways

	Utilisable protein as % of total energy Method of determination			
	NPU (rat)	NPU (child)	Protein score	Average
Maize	4.7	4.8	5.4	5.0
Millet	5.3	5.3	7.7	6.1
Rice	4.9	6.1	6.5	5.8
Wheat	5.9	5.2	5.6	5.6

Protein quality. The protein requirement of 1.27 g kg^{-1} d^{-1} is in terms of milk protein, which is almost 100% utilised when fed at or below the requirement level[26]. In fact, at the age when children develop kwashiorkor they are not fed on milk alone, and so this figure has to be corrected for the quality of the dietary protein. There is much difference of opinion about methods of determining protein quality[32], and, in particular, whether biological values determined on rats are applicable to man. In theory, results on the rat should underestimate quality, because the rat is growing faster than the child. Protein quality depends on the proportion and pattern of the essential amino acids. The essential amino acid requirement for maintenance is relatively low, because recycling of these amino acids is extremely efficient[33,34]. For growth, however, the essential amino acids have to be provided by the diet for deposition in tissue protein. The proportion of essential to total amino acids in the diet must therefore in theory be higher the faster the growth rate. Nevertheless, as Table 2 shows, values for the protein quality (utilisable protein content) of cereals measured directly in rats and in children are in good agreement, and agree also with theoretical estimates based on the proportions of essential amino acids in these proteins (protein scores).

For many purposes it is convenient to convert the amount of utilisable protein provided by a diet or foodstuff from units of weight to units of energy, and to express it as a percentage of the total energy. This is the *P/E* ratio. The figures in Table 2 are expressed in this way. For comparison, the *P/E* ratio in breast milk (early lactation) is about 7.5%, falling to 5% at 2.5 to 3 months of age[35].

Energy requirements. Estimates of the energy requirements of young children have changed less than those for protein (Table 1). The energy requirement is made up of three components—maintenance, growth and physical activity, and we now have fairly reliable figures for each of these. It has been found in a number of animal species, including man, that the maintenance requirement—that is the minimum energy intake at which body weight is constant—is about 1.5 times the basal metabolic rate (BMR)[36]. In the 1-yr-old child the BMR is 210–230 kJ kg^{-1}, so that the maintenance requirement may be taken as 330 kJ kg^{-1}. Studies in children recovering from malnutrition and gaining weight very rapidly have shown that the energy cost of weight gain is about 40 kJ kg^{-1} (ref. 37), if it is assumed that the tissue deposited contains normal amounts of protein and fat. This figure includes both the stored energy and the energy cost of synthesis.

More recent work in Jamaica by Spady (unpublished) suggests that this is an overestimate. He obtained a figure of about 20 kJ per g weight gain. This is the lowest yet found, but is still higher than the theoretical cost of peptide bond and fat synthesis. The rate of weight gain in a normal infant at 9–12 months would be about 1 g kg^{-1} d^{-1}. If we accept the conventional estimate of 430 kJ kg^{-1} for the total daily requirement, this would leave 80 kJ d^{-1} for physical activity. Until very recently these components of energy expenditure in infants have not been studied directly. Spady obtained experimental confirmation of the maintenance energy needs of young children with the instrument known as the SAMI, which counts and integrates the total number of heart beats. After calibration in each child it is possible to monitor the total energy expenditure over a whole day without interfering with the child's activities. Very good agreement was found between energy intake from food and the sum of energy expenditure and energy stored during growth. These results suggest that the estimate of the amount expended in physical activity is reasonable, although of course there will be much individual variation.

Table 3 Energy and protein intakes of children aged 1–2 yr in various countries. Individual weighed intakes after weaning

		Average intake per kg body weight	
Country	No. of child days	Protein (g)*	Energy (kJ)
Ghana	30	1.19	360
Guatemala	28	1.16	323
Jamaica	266	1.47	348
Polynesia	72	1.32	295
Thailand	54	0.61	218
Uganda	124	1.28	285

Sources given in ref. 17.

*As protein which is 100% utilised. Utilisation of protein in actual diets assumed to be 60%.

Perhaps the most important conclusion from this "dissection" of energy expenditure is that the maintenance requirement is relatively so large. An intake of about 320 kJ kg^{-1}, which is quite common in children in developing countries (Table 3) is just enough for maintenance, with no margin for growth or extra activity. Quite apart from the physical effects, this limit on intake may also be bad for the child's mental development, as it will reduce exploration, play and social activity.

Individual variation. The estimates which have been given of protein needs represent what have been called 'safe levels'[27]. These are amounts sufficient to meet the needs even of individuals having unusually high requirements, and are obtained by adding

2 standard deviations (+30%) to the average requirement. In fact, this estimate of 15% for the variability between individuals is almost certainly too high[38], so that the safe levels are very safe

For energy, on the other hand, there is the complication that the cause of variation is partly metabolic and physiological, because of differences in maintenance expenditure and growth rate, and partly due to differences in physical activity. The only estimate we can make of the first source of variation is from measurements of basal metabolic rate, of which the s.d. may be taken as 6.5% (ref. 39). That of the second component—physical activity—will presumably be much larger. With energy, to add a margin of safety will mean that those who need less get too much, and the figures quoted in Table 1 are therefore simply the average values for groups of children.

Comparison of intakes and needs

The question originally posed was whether there was evidence of widespread protein deficiency in children. It would be more precise to ask whether there is evidence that protein is limiting. If the total food intake is inadequate there will be a secondary protein deficiency, because protein cannot be utilised if energy is lacking, and extra protein will be of no value. To answer the question, accurate measurements have to be made of the actual food intakes of individual children, and not simply of the family as a whole. Moreover, because of the difficulty of measuring intakes of breast milk, they must be made on children who have been completely weaned. Studies which fulfil these criteria have been made in a number of countries, but even so, when the literature was summarised two years ago[17], the number of child days covered by the measurements was very small in relation to the importance of the problem. With one exception, all these studies showed that in children 1–2 yr old there was a greater deficit in energy than of protein (Table 3).

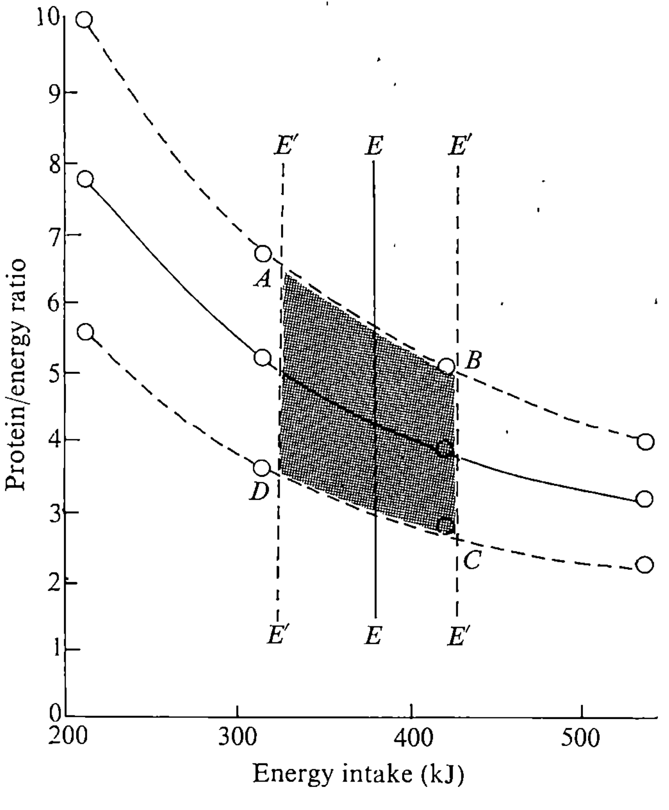

Fig. 1 Ratio of utilisable protein (kJ) to total energy of diets which will provide the average requirement of protein (———) and the average ±2σ (dotted curves), for a 1-yr-old child The top curve represents the "safe level". Vertical lines are estimates of the energy requirement for maintenance + growth + minimal physical activity. The solid line is the average, the dotted lines the average ±2σ The shaded area is the "area of ignorance" resulting from the ranges of individual variation.

Another study in Uganda[40] showed energy intakes 30% below the recommended level, with a marked reduction in physical activity compared with European children. These results are of particular interest because they were obtained in a district where kwashiorkor has long been considered the typical form of malnutrition.

It is obvious that to prevent malnutrition the energy deficit shown in Table 3 must be made up. It then becomes important to define the kind of diets which will fulfil protein needs when enough is eaten to satisfy energy needs. The figures already given show that at 1 yr the protein requirement, expressed as a proportion of the energy requirement, is 5%, so that in theory a diet with a P/E ratio of 5% should meet the child's needs if enough of it is eaten. The problem, however, is complicated by the individual variability of requirements[41].

Figure 1 shows the P/E ratios in diets which will provide the safe level of protein when eaten at different levels of energy intake. The vertical line E–E is an estimate of the minimum energy requirement for health, and represents the requirement for maintenance and growth, with a very small addition for physical activity. This estimate is an average: we do not know the extent of individual variation, so the best we can do is to assume it to be the same as for the BMR, that is about 6.5% (ref. 39). The lines E'–E' represent $E\pm2\sigma(\pm13\%)$. The point A represents the "worst" case, of a child who has the highest need for protein and the lowest for energy. The corresponding P/E ratio is 6.5%. Conversely, the child at C has the lowest protein and highest energy need. A and C then represent the extremes of the range of P/E ratios needed if protein and energy requirements are negatively correlated—a very unlikely situation. Similarly, points B and D represent the range of P/E ratios in a population in which the two requirements are positively correlated. B corresponds to a P/E ratio of 5.0%. There could, of course, be intermediate situations with protein and energy needs being more or less independent in a random fashion (zero correlation). Beaton and Swiss[42] suggested a value of +0.2 for the correlation coefficient. This may be too low, if, as in this case, we are discussing a situation in which physical activity, and thus its contribution to the variance, is low. Protein turnover is a process which is expensive in energy and therefore accounts for a substantial part of the basal energy requirement. The demonstration by Young *et al.*[31] of a parallel between protein turnover rate and requirement has already been mentioned. These considerations suggest that there is likely to be a fairly strong positive correlation (> +0.2) between individual needs for protein and energy.

All we can say so far is that the safe level for the P/E ratio is unlikely to be greater than 6.5% or less than 5.0%, and more research is needed to fill this gap. This is not academic hair-splitting but of great practical importance. Reference to Table 2 shows that the P/E ratios of cereals fall within the likely range of the safe level. The suggestion that cereals without any supplement might provide safe amounts of protein for weaning children may be unacceptable to many. Begum *et al.*[43] have shown that normal growth could be sustained in 2–3-yr-old children by diets in which wheat or rice were the sole sources of protein, but up to now this has not been demonstrated for younger ages. A more precise knowledge of the safe P/E ratio would enable us to assess the significance of the relatively small differences between the cereals; for example, is the somewhat lower value for maize related to the clinical impression that kwashiorkor is commoner in areas where maize is the staple food? The main disadvantage of cereals, and particularly maize, as a principal food for young children is, however, that their bulkiness when cooked makes it difficult for the child to eat enough to satisfy his energy needs, unless—as is unlikely—he has very frequent meals.

The effect of infections

The estimates of protein and energy requirements proposed by FAO/WHO[27] are for healthy people. Even before their latest report was published, its recommendations on protein requirements were described by the Protein Advisory Group of the UN as inadequate, on the ground that no allowance was made for the effect of infections. The argument was that since infections cause a loss of body nitrogen, a high protein supplement is needed to make good these losses. We do not know what is the extent of the nitrogen loss in conditions of real life (as opposed to a metabolic ward). The loss of nitrogen must, however, be accompanied by a loss of energy stores because of the reduced food intake and the extra heat production caused by fever. The extent of these losses is again unknown, but perhaps the problem can be tackled in a different way. The child with an infection ceases to gain weight and very often loses weight. We may then ask, what is the diet necessary for him to catch up and regain his correct weight after the infection is over? If the child has been at constant weight for a month, he needs to gain at twice the normal rate for the next month in order to catch up. Table 4 shows the average *P*/*E* ratio needed for different rates of catch-up. Whitehead[44] has published a similar table, using a different value for the energy cost of growth. Even at rather high growth rates, the required increase in protein content is not very great. Whitehead and his colleagues in Gambia (personal communication) have studied the interrelationship of three factors in rural children: food intake, weight gain and the number of days per month that the children were ill. A higher food intake, derived from the usual diet only, with no supplements, had a very clear effect in counteracting the growth failure caused by infections.

Table 4 Protein and energy requirements for catch-up growth

	Protein ($g\ kg^{-1}\ d^{-1}$)	Energy ($kJ\ kg^{-1}\ d^{-1}$)	*P*/*E*
Maintenance	1.10	440	4.2
Maintenance + normal growth × 1	1.25	465	4.55
Maintenance + normal growth × 2	1.40	490	4.8
Maintenance + normal growth × 3	1.55	515	5.05
Maintenance + normal growth × 4	1.70	540	5.3
Maintenance + normal growth × 5	1.85	565	5.5

Protein increment for normal growth taken as 0.15 $g\ kg^{-1}\ d^{-1}$. Maintenance requirement for energy includes normal level of physical activity. Maintenance requirement for growth taken as 25 kJ per g weight gain.

Another stress is pregnancy. In communities where the women are undernourished a high proportion of infants are born with a low birth weight. Work in Guatemala[45] has shown that increasing the mother's energy intake during the last trimester of pregnancy by a total of 9 MJ or more (as carbohydrate) caused a significant increase in birth weight and a reduction in the infant death rate in the first year of life.

Understanding and prevention

The evidence we have put forward leads to the conclusion that the protein gap is a myth and that what really exists, even for vulnerable groups, is a food gap and an energy gap. This point of view is now fairly widely accepted, particularly in this country, where it has become part of aid policy[46]. What needs to be done to fill the gap is another story. It is important that scientists in this field should make sure that the pendulum does not swing too far. One difficulty is that the controversy about the protein gap, which represents a genuine difference of scientific opinion, is accompanied by another controversy, potentially far more dangerous. This arises from the attitude that research on these nutritional problems is academic, irrelevant and a waste of time; that we know how to prevent malnutrition and therefore what matters is to use this knowledge. Malnutrition is an emotive subject; it is certainly true that there is much that can be done with existing knowledge, and that action cannot wait on the perfectionism of research. But perhaps the story of the protein gap shows the arrogance of supposing that we know the answers, and illustrates the need for a continuing critical examination of the premises on which action is based.

In the long run, the prevention of malnutrition must be founded on an understanding of how the body adapts to different dietary situations, because otherwise concepts such as "deficiency" and "requirement" have no meaning. This is particularly important in relation to marginal states of malnutrition. Nutrition has been called the "Cinderella of the sciences"—justly so, because like Cinderella it is pulled in two directions.

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articles

Lunar temperature and global heat flux from laboratory electrical conductivity and lunar magnetometer data

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Three-layer monotonic electrical conductivity models for the lunar interior to a depth of 600 km are used in conjunction with laboratory measurements of the electrical conductivity of olivine and pyroxene to estimate a temperature–depth profile. The temperatures calculated for depths of 400–600 km are consistent with attenuation of the seismic shear wave. The temperature calculated at a depth of 100–250 km yields a heat flow that is in good agreement with the directly measured lunar heat flow. The temperature, however, is sufficiently close to melting that mascon anisostasy would not be maintained. Thus a better conductor is required at this depth.

THE internal temperature of the moon has been calculated using a set of monotonic conductivity three-layer models derived from Lunar Surface Magnetometer (LSM) data[1] together with laboratory determined conductivity–temperature relationships for two minerals, orthopyroxene (OPX)[2] and olivine (OL)[3], thought to be representative of the interior of the Moon.

The three-layer model is characterised by a core, shell, and crust. The latter has a vanishingly small electrical conductivity. Although the shell and core conductivities can be specified over a broad range of depths, only the conductivities at R_1 (the radius of the core) and R_2 (the outer radius of the shell) are really relevant. This is due to a very strong bias in favour of the outer edge of a layer, because this is where the relevant frequencies tend to be most damped[4]. Resolution tests (Schubert, personal communication) confirm this. Thus the shell conductivity refers to a depth of ~ 150–250 km, depending on circumstances to be discussed here, whereas the core conductivity refers to a depth of ~ 450–600 km. Deeper conductivity cannot be calculated with any confidence from the data in this paper.

For the monotonic three-layer conductivity model, variations in R_1 and R_2 occur because of uncertainty in the estimate of lunar higher order magnetic excitation. The addition to the nominal dipole excitation is primarily from the induced quadrupole moment. The multipolarity arises from higher orders of the field associated with finite wavelengths in the solar wind. For quadrupole and higher order, the direction of the convected wave fronts in the solar wind must be found to determine the pseudoscattering angle, θ. (The term pseudoscattering angle is used to signify that, although the theory of lunar induction is formally similar to Mie scattering, in the case of perfect confinement of the induced field, no field can be radiated. In the restricted theory of lunar induction, however, displacement currents are ignored, and thus only a near field is present.)

This angle, which affects estimates of the higher order induction, is defined as the angle between the phase front vector of the incoming radiation and the lunar radius vector to the site of the LSM. An additional variation in the magnitude of higher order induction arises from uncertainty in the wave phase velocity, U_p. This is generally less than the convected speed of the solar wind. (See refs 5, 6 for the scattering geometry.)

Here we restrict the pseudoscattering angle to $\theta = 150°$, a reasonable approximation for a combination of Alfvén waves propagating down the spiral field direction and tangential discontinuities being convected in the solar wind. Changing either U_p or θ affects R_1, R_2 and the respective conductivities similarly; therefore, we have chosen to vary U_p and maintain θ constant. In general, changes in θ do not produce major changes in the conclusions, except perhaps in the calculation of the heat flux.

Inversion of lunar transfer functions to yield a suite of monotonic three-layer models is based on spherically symmetric plasma confinement theory (SSP)[7]. This leads to an underestimate of electrical conductivity, because, on the Moon, field lines are carried from the front side to the diamagnetic cavity on the antisolar hemisphere, depressing the sunward response[8,9]. This effect is expected to result in a slight upward revision of the temperature estimate when the full asymmetric theory, incorporating the angular spectrum of the wave field, is available.

The dependence of electrical conductivity on temperature was determined in the laboratory using mixed gases to maintain oxygen fugacity at a value within the stability field of the mineral to be measured. The electrical conductivity of the olivine used here is that reported by Duba *et al.*[3] from the Red Sea. Conductivity has been determined with a gas mixture which produces an oxygen fugacity of 10^{-3} Pa (10^{-8} bar) at 1,200 °C. More experimental details and information on gas mixtures may be found in Duba and Nicholls[10]. The OPX conductivity was determined up to 1,400 °C on a sample of the Bamle enstatite[2] previously used for shock compression measurements[11]. OPX_1 refers to measurements for $10^{-3} \leqslant f_{O_2} \leqslant 10$ Pa at 1,200 °C and hydrostatic pressure of 0.5 GPa; OPX_2 refers to measurements for $f_{O_2} = 10^{-3}$ Pa at

1,200 °C and hydrostatic pressure of 0.1 MPa. The electrical conductivity of the enstatite fits the formula

$$\sigma = \sigma_0 \exp(-\varepsilon/kT).$$

A change in the conduction mechanism at ~1,300 °C in the Bamle OPX is suggested by a change in σ_0 from ~ 10 to 30 MS m^{-1} and the activation energy, ε, from 1 to 3.2 eV.

Deep conductivity

Figure 1 shows the temperature at a depth between 450 and 600 km using OPX_1 (lower trapezoid), and OPX_2 and OL (upper trapezoid). In the latter case the temperatures derived from the two minerals are indistinguishable. Both the vertical and horizontal spreads are associated with the influence of higher order modes in the lunar excitation, which affects the calculation of temperature. The variations are due to changes in the assumed value of U_p where $200 \leqslant U_p \leqslant 400$ km s^{-1}, changing the radii within the range $1,200 \leqslant R_2 \leqslant 1,280$ km and the electrical conductivity between $1.6\times10^{-3} \leqslant \sigma \leqslant 1.8\times10^{-3}$ S m^{-1}. The lower trapezoid shows a temperature range of only about 30 °C. All temperatures for R_1 are within ~ 100 °C, or less, of the Ringwood–Essene solidus[12]. This close approach to the solidus is entirely consistent with the attenuation of seismic shear wave depths variously estimated to lie between 500 and 800 km (ref. 13). From calculations of Schubert *et al.*[14] using a lunar model with an isothermal Fe core, convecting shell, and conducting lithosphere, the temperature at the depth discussed here is also consistent with those shown in Fig. 1 based on electrical conductivity.

Corrections to the data for diamagnetism and asymmetric induction would have opposite effects, the former to lower the estimated temperature and the latter to raise it. Diamagnetism requires a pressure gradient which balances the Lorentz force[15]. The slowing of the bulk flow of the solar wind ions at the Apollo 12 site has been attributed to interaction with the remanent field[16]. The slowing of the flow corresponds to the loss of ~ 19% of the free stream flow energy. This effect is the only report from lunar data from which evidence of a pressure gradient might be inferred. The effect of asymmetric induction is of the same order at high frequencies[17].

Although a final assessment of the two effects cannot be made at present, the close correlation of Apollo 12 and 15 data suggests, in any event, that diamagnetism is not important. It could have a role only at Apollo 12, since the interaction with the solar wind is weak or nonexistent at Apollo 15.

For completeness, we have also included data in Fig. 1 from Dyal *et al*[18]. The temperature extremes are calculated from their conductivity estimates using OPX_1. Their 'mean' is shown as the dashed line approximately bisecting their extremes, shown by the shaded region. The temperature profile from the present work is generally consistent with their 'mean', although their estimate of the near-surface temperature is sufficiently low to perhaps satisfy a single mineral composition Moon without involving the problem of mascon anisostasy discussed in the next section. Their deep temperature is depressed somewhat from our estimate, but sufficiently near the solidus to still infer the possibility of shear wave attenuation. The most recent analysis of transients by Dyal *et al.*[19] shows a temperature profile, however, which exceeds the Ringwood–Essene solidus in the outer 500 km of the Moon.

Shell temperature and mascon anisostasy

The three-layer model gives two internal temperature estimates for a specified U_p. Varying U_p between the limits $200 \leqslant U_p \leqslant 400$ km s^{-1} and fixing θ = 150 ° as before, the outer edge of the shell is $1,520 \leqslant R_2 \leqslant 1,600$ km. The corresponding electrical conductivity is $2.9 \times 10^{-4} \leqslant \sigma \leqslant 5.5\times10^{-4}$ S m^{-1} and the temperature is $1,110 \leqslant T_2 \leqslant 1,210$ °C. These values define the two trapezoids to the left side of Fig. 1. The conductivity at depth R_2 is determined strongly by the higher frequencies in the solar wind. Thus higher order modes are here more important than for the conductivity at R_1. For example, for θ = 180° and U_p = 200 km s^{-1}, R_2 = 1,660 km—a depth below the surface of only 80 km. We believe that real resolution at this depth is not attainable without higher frequency data. It is interesting to note that as the position of R_2 moves out with change in induction geometry, the electrical conductivity estimate is depressed, thereby tending to hold the thermal gradient fixed.

From Fig. 1 it is evident that the temperature calculated from OPX_1 is within 100 °C of the solidus of the model lunar pyroxenite of ref. 12. This proximity places a severe constraint on the rigidity of the lithosphere required to maintain the anisostasy of the circular mare mascons[20]. Although the thickness of the lithosphere is uncertain and depends on the viscosity and thermal gradient, a thickness of at least 200–300 km is commonly assumed. We note that, even if the temperature estimate were reduced by estimations based on a less extreme portion of the trapezoids which encompass all the temperature estimates, little would be gained with regard to the rigidity problem. The barred portions of the trapezoids are used to denote that the temperature estimates are potentially in error because of the mascon problem, and may require downward revision. As we shall emphasise later, this implies a compositional change at this depth, but in turn raises difficulties with the heat flux from the Moon.

Global heat flux

A preliminary estimate of global heat flux can be obtained from the previous calculation of temperature at depths of 150–200 km. We have found that this is consistent with the direct heat

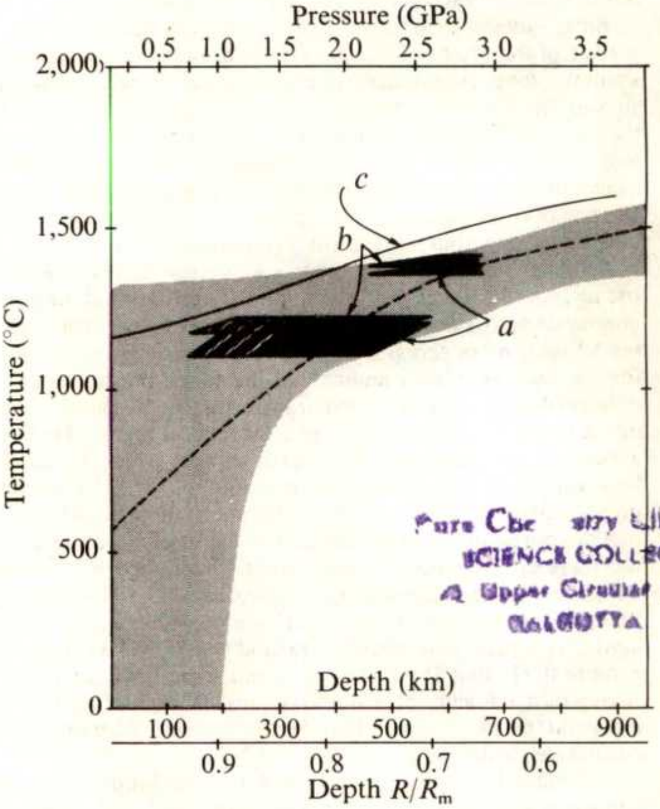

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Fig. 1 Lunar temperature estimates from conductivity models *a*, orthopyroxene (OPX_1; ref. 2); $\sigma_0 = 3 \times 10^7$ S m^{-1}, ε = 3.32 eV for T > 1,327 °C; σ_0 = 2.03 S m^{-1}, ε = 1.05 eV for T < 1,327 °C. *b*, orthopyroxene (OPX_2; ref. 3); for T > 1,327 °C the same as in *a*, σ_0 = 3.49 S m^{-1}, ε = 1.14 eV for T < 1,327 °C; olivine[3]; σ_0 = 17.4 S m^{-1}, ε = 1.34 eV for T < 1,320 °C. *c*, Solidus (ref. 12). Solid areas, obtained from three-layer monotonic model of Sonett *et al.*[1]; shaded area, obtained from nightside and geomagnetic tail-lobe limits, based on OPX_1 (ref. 18): obtained from ———, tail-lobe mean, based on OPX_1 (ref. 18).

Table 1 Thermal gradients calculated for varying U_p and $\theta = 150$ °C in the three-layer monotonic conductivity model

U_p (km s^{-1})	R_2 (km)	T_{OPX_1} (°C)	T_{OPX_2} (°C)	T_{OL} (°C)	dT/dR ($10^5 \times$ °C km^{-1})		
					OPX$_1$	OPX$_2$	OL
400	1,519	1,210	1,240	1,230	5.6	5.7	5.7
300	1,537	1,190	1,220	1,210	6.0	6.0	6.1
200	1,600	1,140	1,140	1,140	8.2	8.4	8.4

flux measurements of Langseth *et al.*[21] but is possibly too high, for reasons given in the previous section. Table 1 shows thermal gradients calculated assuming a linear increase of temperature with depth between the surface and R_2. The thermal gradients are insensitive to variations in the relative amounts of OPX and OL. Thermal gradients (dT/dR) from Table 1 are used to construct Table 2, which shows estimates of heat flux, incorporating the variable U_p. To calculate heat fluxes in Table 2 we use an average thermal conductivity of 4.18×10^{-2} W cm^{-2} °C^{-1}, a reasonable mean between a Hortonville dunite (3.64×10^{-2} W cm^{-2} °C^{-1}) and a Bushveldt pyroxene (4.93×10^{-2} W cm^{-2} °C^{-1})[22]. Langseth *et al.*[21] report a range of heat fluxes from 2.99–3.59 μW cm^{-2} at the Hadley–Appenine site, with their best value being 3.31 μW cm^{-2}. Assuming that the most likely value for phase velocity is $200 \leqslant U_p \leqslant 300$ km s^{-1}, as seems to be the most reasonable from arguments based on the solar wind data and studies of the lunar response, the magnetometer derived heat flux is 2.5–3.5 μW cm^{-2}. This is a global or large area average and is not dependent on local variations, but is model dependent, varying with U_p and compositional assumptions.

Discussion

Subsidence of mascons may have taken place over a long period of time, but this is uncertain; they are now below the mean surface of the Moon. There is some evidence that at least some shallow zone moonquakes are connected with ongoing subsidence. Lunar transient events are thought to correlate with the ringed maria and with shallow zone quakes[23]. The Apollo 15 magnetometer anisotropy may require a dynamic process, suggesting a model of circumferential cracking at great depth. In spite of these indications of settling, the mascons seem to be anisostatic, and thus some crustal rigidity is required.

Actually the issue of mascons and isostasy seems unsettled because of the combination of lateral positive and negative anomalies across Serenitatis, for example. Hulme[24] produced a model with mass excess overlying mass deficiency to account for the negative gravity anomaly at the edges, but his model is only qualitatively correct and transforms the global problem into a problem of the stability of a more local region. He finds a negative anomaly only one-fourth of that which he quotes for Muller and Sjogren[25], and he states that it is difficult to provide quantitative consistency without changing his (Hulme's) model appreciably. Other models for the negative anomaly at the mare edge suggest a mass excess surrounded by a mass-deficient ring, correspondingly to crater excavation followed by filling[26,27]. Current mascon models are non-unique, but in the light of this paper, the seismic data, and heat flow data, we think it more likely that the mascons exhibit large scale anisostasy. A ring of mass-deficient material is consistent with this, though it should be pointed out that Hulme's model cannot yet be conclusively ruled out.

If a rigid lithosphere is required on the Moon, then the temperatures estimated from the LSM and the electrical conductivity data on OPX and OL seem to be high, and the presence of a material having a higher electrical conductivity at a given temperature is required in the outer 200±50 km of the Moon. We think that the problem of the source of the mare basalt is, as yet, too poorly understood to be able to infer a composition. The situation with the electrical conductivity–temperature functions for basalts is equally poorly understood.

Nevertheless it is worth noting that the electrical conductivity of terrestrial basalts, summarised by Duba and Lilley[28], indicates that a lower temperature at a depth of ~ 250 km may result if the outer shell of the Moon were composed of basalt. The inferred depression of the temperature would strongly affect the estimates of dT/dR and the heat flux (H) shown in Tables 1 and 2. It should be noted that all electrical conductivity measurements of basalts have been made under conditions potentially favourable to oxidation of Fe^{2+} to Fe^{3+}. Since Duba and Nicholls[10] have shown that the conductivity of olivine with Fe^{3+} may be increased by as much as three orders of magnitude, investigations of the electrical conductivity of basalt in conditions of controlled oxygen fugacity are now under way.

Table 2 Global heat flux μW cm^{-2} estimated from Table 1

U_p(km s^{-1})	H_{OPX_1}	H_{OPX_2}	H_{OL}
400	2.35	2.40	2.38
300	2.51	2.57	2.56
200	3.41	3.51	3.52

Because of the difficulties with compositional models and electrical conductivity functions, we can only say that a substantial depression of the temperature at R_2 is required to satisfy the mascon-viscosity requirement. A guess is that the outer shell temperature at R_2 is between 600 and 1,200 °C, with prejudice towards the lower value. This would immediately depress the estimated global heat flux by about a factor of two. Since Langseth *et al.*[21] inferred a global radionuclide concentration of about twice the chondritic value, the arguments given here would make the two approximately equal. These comments are based on the assumption that the Moon is at present in thermal equilibrium, a condition achievable in the present epoch only if strong convection has taken place.

Nakamura *et al.*[13] infer a thermal gradient of $2 \leqslant dT/dR \leqslant 5$ °C km^{-1} at a depth of 60 km (the bottom of their zone 1). This is a small thermal gradient, but their upper limit is consistent with a lowered global heat flux. It should be noted that the determination of the thermal gradient for the several experiments discussed here is for widely differing depths. We have assumed here that dT/dR is constant from the surface to R_2, whereas the thermal gradient probably diminishes with depth. Thus Table 2 provides upper bounds for dT/dR. The heat-flow experiment measures the heat flux in the regolith, and therefore requires a secondary experiment to determine the thermal conductivity of the regolith, which cannot be compared directly with that at 150–250 km. It is likely that the radionuclide concentration is non-uniform and that the region near R_2 contains a significant fraction of the lunar radionuclide concentration. The outward migration of the radionuclide concentration inferred by these comments means that dT/dR would be increased in the layer very near to the surface, but possibly decreased near R_2. Thus the question of the correct thermal gradient, and to which depth it refers is incompletely explored.

Conclusions

If the Moon is assumed to have a composition of OPX, OL, or a mixture of the two at a depth of 450–600 km, the temperature calculated from magnetometer data is near to the solidus of Ringwood and Essene[12]. This high temperature is in accord with the evidence from seismic data of the attenuation of shear waves at a depth of 500–800 km. A temperature from nearer to the surface, at a depth of 150–250 km, is found by assuming that the composition at shallow depth is similar to that assumed for 450–600 km. There the temperature is even closer to the melting curve. The thermal gradient inferred from the shallow temperature is consistent with the values found by Langseth *et al.*[21], but cannot provide the lithospheric rigidity needed for support of the mascons. A reduction in the estimate of lithospheric temperature can be made only if a more conducting material is present at the depths of about 150–250 km. At the same time this reduction in the lithospheric temperature depresses the calculated heat flux. Depending on the material composition, the temperature estimate using the results of Langseth *et al.*[21] could be reduced by a factor of nearly two. If heat-producing radionuclides were concentrated in the outer 60 km, the thermal gradient derived in this paper would be more in accord with that estimated by Nakamura *et al.*[13]. The reconciliation of dT/dR at 60 km inferred from the seismic data and that inferred from the conductivity data, requires further study to find the curvature in the thermal profile, that is d^2T/dR^2.

Substances which are consistent with geophysical constraints for the lithosphere, derived from the magnetometer data, include possibly basalts, but this may be inconsistent with geochemical evidence. Recent electrical conductivity measurements on the albite end member of the plagioclase series indicate that plagioclase in the lunar upper mantle would yield lower temperatures than olivine and pyroxene[29]. Petrologic models which envision an ilmenite-rich layer[30] in this region would conceivably also allow higher electrical conductivity at lower temperatures. The available magnetometer data cannot provide details of the conductivity profile; but the requirement that the conductivity–temperature dependence be greater, that is, higher conductivity for the same temperature, could easily mean a non-monotonic conductivity profile. It is unlikely, noting the requirement for a change of composition to more conducting material, that the conductivity–depth relation is monotonic. Thus the alternate model of the interior conductivity, with a narrow region of high conductivity, remains electrically valid[31] and is a plausible petrological and geophysical model.

Finally we note that in the region between R_1 and R_2, the thermal gradient, $dT/dR \sim 1\ ^\circ\mathrm{C\ km^{-1}}$, whereas the adiabatic lapse rate at these depths is $\sim 10^{-2}\ ^\circ\mathrm{C\ km^{-1}}$. It is unsafe to speculate on the possibility of solid state convection at this depth because of the paucity of data, but the evidence is not favourable. Convection at greater depths is still a possibility. It should be noted that uncertainty about the lithospheric composition would even apply if there were a little water present. Although there seem to be compelling geochemical arguments against this, it would be unsafe to draw an absolute conclusion regarding H_2O. The effect of water on the electrical conductivity has not been fully investigated.

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Cytological detection of mutagen–carcinogen exposure by sister chromatid exchange

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A staining technique that detects sister chromatid exchanges (SCEs) has been used to examine the response of chromosomes in cultured Chinese hamster cells to a wide variety of mutagens–carcinogens. The test gives a very sensitive and rapid method for detecting chromosome mutagenicity of chemical agents and provides a powerful new method for detecting environmental mutagens.

SISTER chromatid exchange (SCE) involves a symmetrical exchange at one locus between sister chromatids, which does not result in an alteration of overall chromosome morphology. Such exchanges were originally demonstrated by Taylor[1] in autoradiographic studies of plant chromosomes and were seen as switches in radioactive label between chromatids at the second (M_2) mitosis after ^{3}H-thymidine labelling at the S phase of the M_1 cycle. Later studies showed that many of the SCEs observed in autoradiographic experiments were induced by the endogenous radiation from the incorporated tritium[2]; that the frequency of SCE was increased in cells exposed to X rays[3], ultraviolet light[4,5] and certain chemical mutagens[6]; and that a significant proportion of gross chromatid aberrations (including deletions) seemed to involve an SCE event at the site of aberration[7].

Techniques have been developed which distinguish between sister chromatids without using radioisotopes and autoradiography. These involve exposing cells to 5-bromodeoxyuridine (BUdr) for two rounds of replication so that M_2 chromosomes possess one chromatid unifilarly substituted with BUdr and its sister bifilarly substituted. Such chromatids stain differentially with Giemsa[8], the fluorochrome Hoechst 33258[9] (and various other fluorescent dyes, for example, acridine orange and quinacrine), or a combination of fluorochrome plus Giemsa—the FPG technique of Perry and Wolff[10]. At least a proportion of SCEs observed in BUdr-treated cells are induced by the analogue[11], but Latt[12] has demonstrated a fourfold increase in SCE in BUdr-treated human lymphocytes exposed to the chemical mutagen mitomycin C (MMC) at a dose which causes only a low frequency (0.07 per cell) of chromatid aberrations.

We have confirmed Latt's[12] finding and, since the FPG technique provides an easy, rapid and high resolution method for detecting SCE, investigated whether the induction of SCE could provide a simple and sensitive endpoint for assaying mutagenicity in mammalian cells. We now present some of our results obtained with a line of Chinese hamster fibroblasts (CHO) exposed *in vitro* to fifteen mutagens–carcinogens, each in a range of concentrations (doses). Our findings from these experiments, and from preliminary studies on patients treated with cytotoxic drugs, strongly suggest that the SCE test provides a powerful tool for *in vitro* mutagen testing and possibly for monitoring people exposed to known or suspected mutagens–carcinogens.

In all the *in vitro* experiments CHO cells were used and cultured as described previously[11]. Exponentially growing cells were exposed to 10 μM BUdr for two cycles of DNA replication before treatment with colcemid (2 h at 2×10^{-7} M), collection by mitotic shake-off[13], pretreatment for 7 min with 0.075 M KCl and fixation in 3 : 1 methanol–acetic acid. Throughout, all cultures were maintained in the dark or under a yellow safe light to minimise photolysis of BUdr and background SCE[8]. Air-dried preparations were made, stained by the FPG procedure[10] and coded and scored for SCE and chromosomal aberrations by one observer.

X rays

SCE were scored in "harlequin-stained" (Fig. 1) chromosomes at the first mitosis after acute (50 rad min^{-1}) X-ray exposures at various doses. Using synchronously developing cultures (see legend for Fig. 2), the responses of cells exposed in G_1, S and G_2 were determined (Fig. 2). The incorporation of BUdr into DNA enhances its sensitivity to damage by both X rays[14] and ultraviolet light[15]. In our experiments chromatids in G_1 cells were all unifilarly substituted with BUdr at the time of irradiation, whereas half of the G_2 chromatids were bifilarly substituted. Our data demonstrate, however, that the chromosomes in G_2 cells showed no increase in SCE over background at any of the doses, whereas there were significant dose-dependent increases in S and G_1 cells.

Studies on chromatid aberrations induced by certain chemical mutagens[16,17] have shown that cells exposed in G_2 do not yield aberrations at the ensuing mitosis, but do so at the subsequent division, the development of aberrations necessitating an intervening DNA replication between treatment and observation. Somewhat similar, but not identical, findings have also been reported with ultraviolet light[18,19]. With ionising radiations, however, irradiation of G_2 cells results in the formation of aberrations which are seen at the first mitosis after exposure, and although there is some evidence for a small increase in aberrations in irradiated G_2 cells at their second mitosis[19], it is clear that a large proportion of the lesions that develop into aberrations do so shortly after their formation without the need to pass through a replication phase. Our data on the X-ray induction of SCE, however, closely parallel those obtained for aberrations with ultraviolet light and many chemical mutagens, suggesting that the X-ray-induced lesions resulting in SCE are relatively long lived (and are presumably therefore not single strand breaks[20]), become manifest as SCE events as a consequence of DNA replication and reflect a type of recombinational repair process.

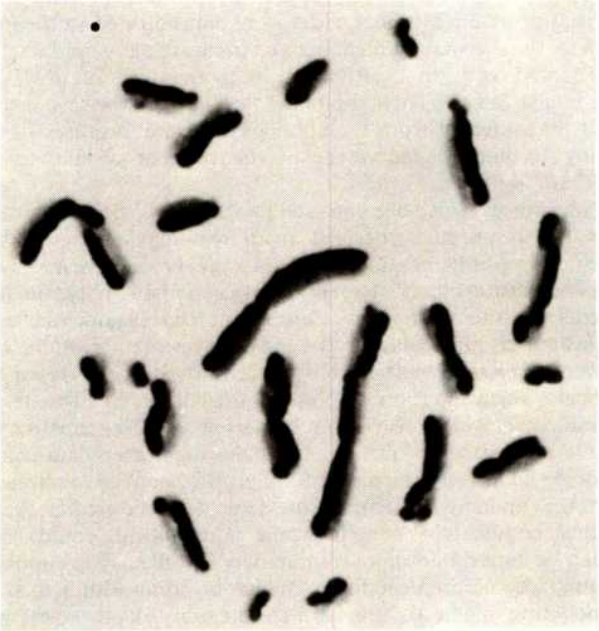

Fig. 1 M2 metaphase cell from a Chinese hamster cell line (CHO) after two rounds of replication in the presence of BUdr (10 μM) and stained with the FPG technique. Note the differentiation between chromatids and the presence of 12 SCEs.

In the context of this report, we emphasise that although there is a linear or curvilinear relationship between X-ray dose and SCE yield in G_1 and S cells, the increased incidence of SCE is minimal in relation to the induced incidence of chromosome and chromatid aberrations and this contrasts markedly with our results on chemical mutagens (see below). For example, at an X-ray dose which doubles the SCE incidence (~400 rad) there is a more than twentyfold increase in aberration frequency. This would imply that the induction of X rays of the kinds of lesions that result in SCE is inefficient relative to those events that result in aberrations, or, that most of these lesions are repaired (and a proportion gives rise to aberrations?) outside the S phase. In any case it seems clear that counting the incidence of SCE does not seem to provide a sensitive system for detecting X-ray exposure.

Chemical agents

The effectiveness of a chemical as a mutagen depends on various factors. Some mutagens are highly reactive with a short half life *in vivo* and may be very unstable in solution so that treatment with such agents, for example, sulphur mustard and to a lesser extent methyl and ethyl methane sulphonates (MMS and EMS) and N-methyl-N-nitro-N-nitrosoguanidine (MNNG), is essentially in the form of an acute or semi-acute exposure. Other mutagens, for example, mitomycin C (MMC), are much more stable and their continued presence effectively results in a chronic exposure. The activity of most agents is very dependent on *p*H and some, for example, cyclophosphamide, are not active *per se*, but are metabolised within the body to give mutagenic metabolites. Much human exposure to environmental chemical mutagens may be considered to be akin to a chronic exposure, so that in the present experiments all chemical agents were introduced into the culture medium at the time of addition of BUdr and the medium was not changed until the cells were sampled 24–36 h later.

Most of the agents selected for study were chemically unrelated, but all were proven (or suspected) mutagens or carcinogens. Many caused considerable delays in cell progression and these delays were assessed for each concentration of chemical in preliminary experiments in which mitotic cells were collected at various intervals after 24 h, and final sample times were then selected to give good yields of M_2 cells. Virtually all control cells sampled after 24 h were in the M_2 division, whereas with sub-lethal doses of many agents, samples collected as late as 36 h contained a high proportion of M_1 cells. These delayed M_1 cells showed a very high frequency of chromosomal aberrations compared with M_2 cells on the same slides (Table 1). It is important to realise therefore that cells scored for SCE at high concentrations represent selected samples which must give un underestimate of the maximum SCE frequency.

The chemical agents studied, and the relationship between initial dose (concentration) and SCE frequency in M_2 cells, are shown in Fig. 3. The alkylating agents, and in particular the bifunctional compounds—MMC and nitrogen mustard (HN_2, Fig. 4)—are extremely potent inducers of SCE in CHO cells, with MMC giving a doubling of SCE freqency at 10^{-8} M. The monofunctional alkylating agents, on the other hand, are generally less effective and show a considerable range in their ability to induce SCEs. This result is consistent with the finding that monofunctional agents are usually less efficient at inducing chromosomal aberrations than their bifunctional analogues[21]. Monofunctional compounds, however, may be more efficient in inducing mutations in bacteria and bacteriophage[21]. It is also notable that of the sulphonates, MMS is a more potent inducer of SCE than EMS, and this is also true for the induction of chromosomal aberrations. Thus, the ability of an alkylating agent to induce SCEs seems to bear no simple direct relationship to its efficiency in inducing point mutations in bacteria, but is more closely related to its ability to induce chromosomal aberrations in eukaryotes.

Quinacrine mustard (QM) produces chromosomal aberrations and is a monofunctional alkylating agent which may also intercalate between the bases in DNA[22]. Other intercalating agents produce frame shift mutations in bacteria as a consequence of distorting the double helix, and QM and the two other intercalating agents we studied, the anti-tumour[23] antibiotic adriamycin and the fluorochrome Hoechst 33258, a dibenzimidazol derivative[24], are also very effective inducers of SCE, with adriamycin being a very efficient inducer of chromosomal aberrations[25]. Chromosomal aberrations are also induced by the potent carcinogen[26] and mutagen[27] 4-nitro-quinoline 1-oxide (4NQO), which is also a very efficient inducer of SCE.

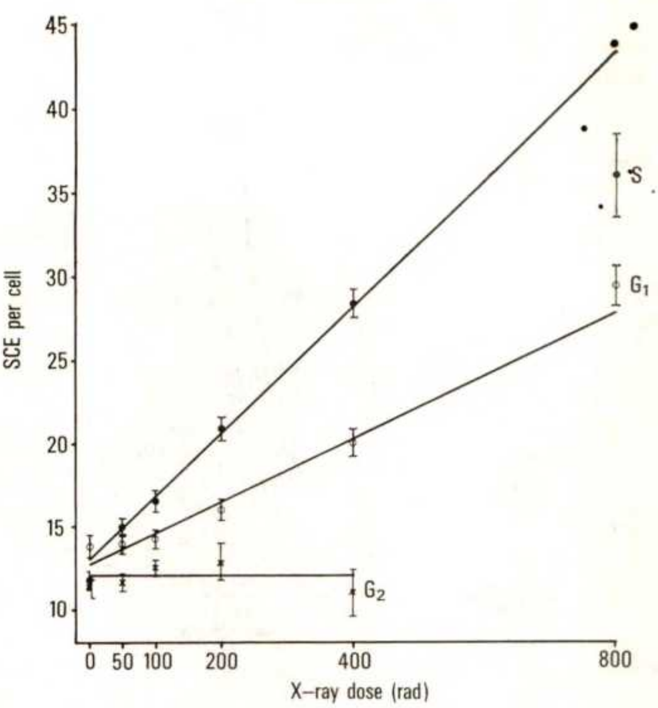

Fig. 2 The relationship between X-ray dose and SCE frequency in M_2 cells exposed to radiation in G_1 (○), S (●) and G_2 (×) of the second cell cycle. All irradiations were carried out on cells which had completed at least one round of replication in BUdr, using a Siemens Stabilipan X-ray set giving 50 rad min^{-1} at 250 kV and 15 mA after filtration with 0.8 mm Su, 0.25 mm Cu and 1 mm Al. Cells exposed in G_2 were treated with colcemid 90 min after exposure of asynchronously developing populations and collected by mitotic shake-off 2.5 h later. For G_1 and S exposures, cells were synchronised by mitotic shake-off after one cell cycle of culture in the presence of BUdr (11–12 h). Serial shake-offs were performed every 15 min and cell samples with a mitotic index ⩾ 98% were collected and pooled, on ice, to give sufficient cells for replating at 37 °C and irradiation. Cells were irradiated 90 min (G_1) or 7.5 h (S) after plating and cell stages were checked by autoradiography of parallel ^{3}H-thymidine labelled cultures. G_1 and S cells were maintained in BUdr medium and exposed to colcemid before collection 17 h and 13 h after irradiation. Sampling times were adjusted to take account of mitotic delay and SCEs were scored in metaphase cells with harlequin-stained chromosomes characteristic of cells completing two rounds of replication in the presence of BUdr (Fig. 1). The lines in the figure were drawn by eye and each point represents a mean ± s.e. from 50 cells.

Figure 3 shows that two of the fourteen chemical agents studied induced no SCE. Maleic hydrazide (MH), which is widely used as a herbicide, is a potent inducer of chromosomal aberrations in plant cells when applied in solution at low *p*H[28]. It has never been reported to cause chromosome damage in mammalian cells and in our experiments, where *p*H was maintained at 7.2 using HEPES buffer, it induced neither aberrations nor SCE. The other compound which had little effect on SCE was the antineoplastic[29] agent cyclophosphamide. In itself, cyclophosphamide is ineffective as a cytostatic or chromosome damaging drug, but it is metabolised in the liver to give a very active bifunctional alkylating intermediate[30]; its inefficiency in producing aberrations and SCE in our *in vitro* system is therefore hardly surprising.

Table 1 Frequencies of chromosome aberrations in 34-h samples of cells exposed to 10^{-7} M mitomycin C (50 cells per sample)

Treatment	Chromosome aberrations				Chromatid aberrations		
	Dicentrics	Rings	Deletions	Gaps/Breaks	Rings	Isochromatid	Interchanges
MMC only	1	1	—	1	—	4	5
MMC+BUdr M_2 cells only	—	6	—	—	2	—	4
MMC+BUdr M_1 cells only	1	4	1	7	1	2	9

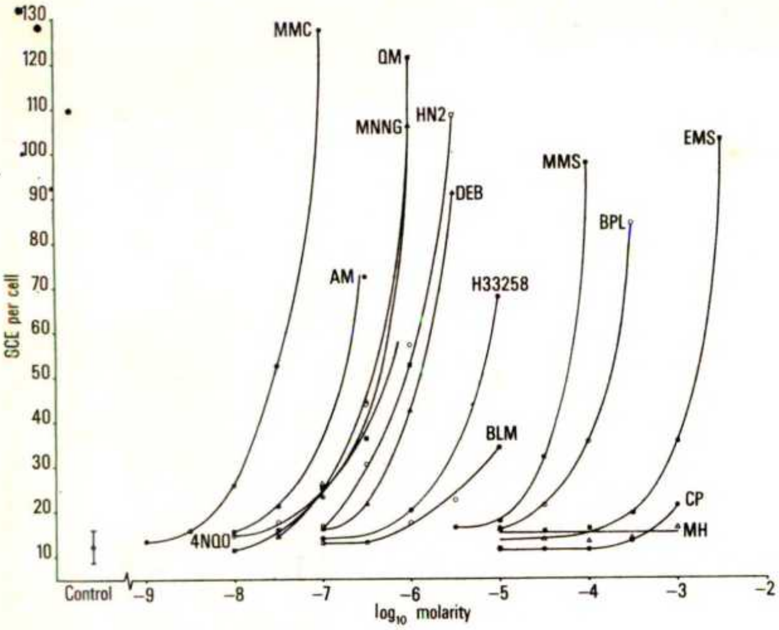

Fig. 3 Dose–response curves for SCE incidence in CHO cells against log of initial concentration of chemical agent, for the fourteen chemical agents studied. All chemicals were added to culture medium at the time of addition of BUdr and the medium was not changed until collection (see text). Those agents that were insoluble in water were first dissolved in a small volume of ethanol before dilution in tissue culture medium and all dispensing was completed in 8 min. Collection times were adjusted to take account of mitotic delay and cultures were maintained at 37 °C. SCEs were scored in harlequin-stained chromosomes of second division cells, and each point on the graph represents a mean frequency of SCE from 20 cells (~400 chromosomes); standard errors are not shown since for all points they were less than 5% of the mean frequency. MMC, Mitomycin C; AM, adriamycin; MNNG, N-methyl-N-nitro-N-nitrosoguanidine; QM, quinacrine mustard; 4NQO, 4-nitroquinoline 1-oxide; HN2, nitrogen mustard; DEB, diepoxy-butane; H33258, Hoechst 33258; BLM, bleomycin; MMS, methylmethane sulphonate; BPL, β-propriolactone; EMS, ethyl methane sulphonate; CP, cyclophosphamide; MH, maleic hydrazide.

All the tested agents which induce SCE also induce chromosomal aberrations, and an important practical question is whether these agents produce significant increases in SCE at doses which induce only a negligible or low aberration frequency. We have examined this question by scoring for aberrations at doses which give a doubling of SCE incidence, and for doses which result in a tenfold increase in SCE. The results are summarised in Table 2. In considering these data it must of course be remembered that SCE can only be scored in M_2 cells and, as already indicated (Table 1), the frequency of chromosomal aberrations is often higher in M_1 cells. Some of the aberrations were induced by the BUdr itself[31] (Table 1) and most of the chemical agents were found to induce chromatid-type changes. Most of the chromosome-type aberrations in the M_2 cells listed in Table 2 were "derived"[32] by duplication from chromatid-type aberrations produced in the first cell cycle. The data in Table 2 show that for all but one (bleomycin) of the twelve mutagens–carcinogens studied, a dose of mutagen that gives a highly significant doubling of the incidence of SCE produces only a minimal and, with the small number of cells scored, barely noticeable effect on the incidence of chromosomal aberrations. Doses giving tenfold increases in SCE gave increased, but still very low, aberration frequencies.

The scoring of chromosomal aberrations requires a high

Table 2 Total chromosomal aberration frequency and total SCE in 20 M_2 cells scored at each of two doses for each chemical agent

Chemical	Chromosome aberrations (B″)			Chromatid aberrations (B′)				Total B″+B′	Total SCE
	Dicentrics	Rings	Deletions	Gaps/breaks	Rings	Isochromatid	Interchanges		
Control									
BUdr only	1	1		1				3	244
AM 3×10^{-8} M				1		1		2	430
3×10^{-7} M				++	++	++	++	++	1,444
EMS 3×10^{-4} M		2	2					4	382
3×10^{-3} M			1	1			1	3	2,054
MMS 3×10^{-5} M		1						1	644
3×10^{-4} M		1						1	1,952
MMC 10^{-8} M		2	3	1				6	512
10^{-7} M								0	2,555
HN_2 3×10^{-7} M								0	612
3×10^{-6} M		2				1		3	2,175
4NQO 10^{-7} M								0	462
10^{-6} M			1					1	1,140
MNNG 10^{-7} M		1	2					3	528
10^{-6} M		4		3	2		3	12	2,124
DEB 3×10^{-7} M			1			1		2	403
3×10^{-6} M		4						4	1,818
BLM 3×10^{-6} M				++	++	++	++	++	451
—	—	—	—	—	—	—	—	—	—
QM 10^{-7} M								0	506
10^{-6} M		1	4					5	2,422
CP 10^{-3} M		1	1					2	424
—	—	—	—	—	—	—	—	—	—
BPL 3×10^{-5} M		2						2	425
3×10^{-4} M		3	1				1	5	1,668

The two doses selected were those which resulted in an approximate doubling and an approximate tenfold increase in SCE. With all agents except bleomycin, a doubling dose for SCE resulted in no obvious increase in chromosomal aberrations in the M_2 cells scored, and, in most cases, a dose giving a tenfold increase in SCE gave only a very much smaller increase in chromosomal aberrations. With both bleomycin and adriamycin at 3×10^{-7} M, there were too many chromatid-type aberrations present for accurate scoring, and this is indicated by ++.

level of skill and is very time consuming, since even at fairly high doses of mutagen the analysis of up to 100 cells or so per dose, which may involve several hours at the microscope, may be necessary to obtain significant results. Our findings show that through determining SCE frequencies it is possible to detect significant effects of mutagens on chromosome structure at doses that are usually very much lower than those necessary to produce marked increases in chromosomal aberrations. Moreover, the scoring of SCEs requires little training and is extremely rapid, so that, for example, the detection of a doubling of background incidence, at the 5% level of significance, requires the scoring of no more than 10–20 cells, which takes a matter of a few minutes.

In vivo studies on man

We have shown[33] that the incidence of SCE in human blood lymphocytes is relatively constant between individuals, and is independent of age and sex. The notable exception here is the very high SCE incidence in cultured cells from patients with Bloom's syndrome[34], an increase which is not evident in cells from patients with other inherited chromosome instability syndromes[33,34]. This stability, and the very high sensitivity of what we have referred to as the SCE test, has therefore led us to look for increases in SCE frequencies in peripheral blood lymphocyte chromosomes of patients undergoing therapy with cytotoxic drugs. Only preliminary results are available and our detailed findings will be presented elsewhere. But it is clear from our first results on patients treated with adriamycin that significant increases in SCE are to be observed in blood cells sampled 24 h after injection with this drug, at dose levels which produce only a negligible frequency of chromosomal aberrations in these cells. It seems possible therefore that we may be able to use SCE incidence to detect significant exposures of individuals to mutagens–carcinogens.

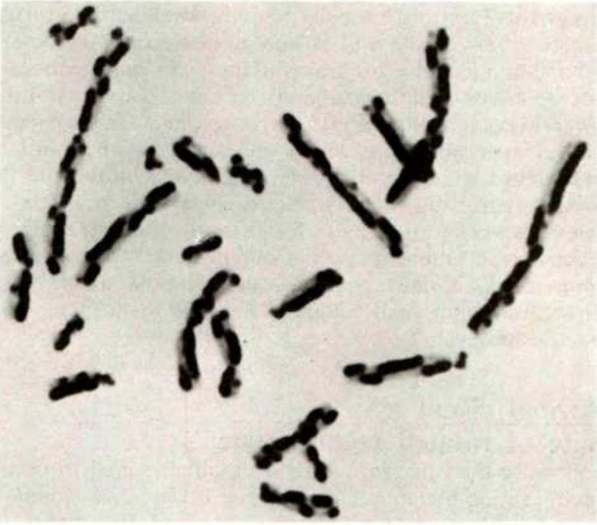

Fig. 4 M2 CHO cell after exposure to BUdr and HN_2 (3×10^{-6} M) throughout the two cell cycles before sampling. Note the approximately tenfold increase in SCE relative to the example from controls in Fig. 1.

Implications and applications

Our results show that the marked increase in incidence of SCE in cells exposed to a wide variety of chemical mutagens is associated with very much smaller increases in chromosome aberration yields, whereas the converse holds in cells exposed to X radiation. Chromosomal aberrations are of course mutational events, although many will be expressed as dominant lethals in germ cells. A very relevant question therefore is whether SCE events are themselves associated with mutational changes? This question is difficult to answer, but we believe that at least some sister chromatid exchanges may be associated with mutation. Our reasons for this belief are based on two arguments. First, SCE is often seen to have occurred at the site of chromatid breakage, or intrachange resulting in duplication and/or deletion. It is evident therefore that incomplete or abnormal SCE events do occur, and that these certainly result in aberrations and mutations. Second, the exchange event that results in SCE involves recombination between parent and daughter polynucleotide strands at sites of transient discontinuity in both parental strands[11]. Our data suggest that this recombination repair process[36] takes place at the time of DNA replication and it seems not improbable, as has been demonstrated in bacteria[37] and meiotic cells of yeast[38], that mutation originates as an error in the recombination repair process following mispairing and base deletion or insertion.

Our data show that SCE frequencies are far more sensitive indices of chromosome damage than frequencies of gross chromosomal aberrations, and that the new techniques to detect them have provided a simple, reliable and highly sensitive method for assaying the chromosome mutagenicity of environmental agents. Only further work will establish whether these techniques will provide a good system for monitoring human exposure, but we suggest that the *in vitro* SCE test on human or other mammalian cells should form part of any environmental mutagen testing programme.

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Polarity of structure and of ordered nerve connections in the developing amphibian brain

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Tectal polarity for retinal connections remains reversible long after the anatomical pattern of neural structures has been determined. Cells in the diencephalon seem to control this polarity. Following certain embryonic operations, the diencephalon developed behind the tectum. In such cases, the polarity of the retino-tectal projection was reversed.

THE amphibian retino-tectal projection has been widely used for studying the mechanisms governing selective nerve connections. One important fact which has emerged from the study of this system is that critical events occuring early in ontogeny determine the axial polarity of the retina with respect to its connections to the tectum, the primary visual centre. When an eye in a stage 32 *Xenopus*[1] is rotated by 180°, cells in the original anterior pole of the retina connect to the posterior pole of the tectum, as they would have done if left undisturbed, and the animal's visual world is accordingly rotated by 180°. If a similar operation is performed some 7 h earlier (Stage 28 in *Xenopus*), the animal develops normal visuo-tectal connections[2-5], because of repolarisation of the eye in the orbit.

It has been conjectured[6] that an equivalent determination of axial polarity occurs within the tectum, to which retinal cells later connect through their optic nerve fibres. The time of tectal polarisation is not known, for the only attempted rotation experiments on the tectum comparable with those on the embryonic eye did not give clear results[7]. If such tectal polarisation does take place, it is likely that the information for this, whatever its nature, may originate from a local group of cells, as has been demonstrated for the control of spatial patterns of cellular differentiation early in embryonic development[8,9].

Here we report briefly on the results of an investigation directed at these questions. Our findings indicate that such polarising information can originate from a group of cells outside the tectum, namely, those which are subsequently to form the diencephalon. They also suggest that tectal cells do not acquire permanent positional labels before optic innervation. Detailed reports, including autoradiographic studies of histogenesis and electrophysiological evidence for the presence of normal synapses in rotated tecta, will be presented elsewhere.

Embryonic operation

The presumptive midbrain regions of 83 embryonic *Xenopus* were cut out and replaced after rotation by 180°. Most of the operations were carried out at stages 21–24, or approximately 22–26 h after fertilisation, with some at stage 37, some 24 h before the optic nerve reaches the tectum[10,11]. No differentiation is detectable among cells of the neural tube until midway between these two periods, but earlier work on another species[12] has shown that even well before stage 20 the neural rudiment is a mosaic of regions, each with a strong tendency to develop into a particular part of the brain. We confirmed that this situation obtains also in *Xenopus* by examining embryonic brains following rotation of small pieces in neural plate stages. The dorsal diencephalon (thalamus) and adjoining optic tecta develop from a dorsal section, some 15 to 20 cells square as counted directly through the dissecting microscope, of the stage 22 neural tube (Fig. 1). By varying the anterior–posterior levels of the cuts, but attempting to rotate in each case a similar sized piece, we were able to produce brains with different spatial arangements of these anatomical parts.

Owing to elastic forces resulting from morphogenetic movements and cellular differentiation, the operation on the brain rudiment of stage 37 larvae is difficult. Nevertheless, we have succeeded in mapping two animals after rotating tectal structure at this stage.

We used several criteria for assessing operations as successful. First, we checked visually whether the cut tissue remained in place 48 h after operation. Second, we observed gross derangements of brain anatomy in cases where the rotated tissue had healed properly, and confirmed this in serial histological sections. Finally, when we carried out control experiments in which the cut piece of neural tube was discarded, we observed two classes of results from those animals surviving until metamorphosis. In one class of animals, regeneration had given rise to brain structure and retino-tectal connections indistinguishable from those of unoperated animals, and the other showed atectal brains with forebrain grossly enlarged and the diencephalon joining the brainstem.

Normal visual map in spite of rotated tectal tissue

Following the operation, only 10 animals failed to establish good retino-tectal connections. From the remaining animals, 63 electrophysiological maps of the direct optic projection to the contralateral tecta were obtained during metamorphic climax, using procedures detailed elsewhere[10]. For clarity, only one or two of the several rows of electrode positions plotted are shown for each map presented here. The brains and their associated maps fell into three main categories, which we now discuss in turn.

A parasagittal section of the normal brain is shown in Fig. 2*a*. The diencephalon (D) is seen immediately in front of the midbrain, with its single tectum (T). Figure 2*b* shows a comparable section of a brain where we believe the anterior part of the tectal precursor had been rotated. Viewed from the dorsal surface, a pair of tecta were clearly identifiable on each side, separated by the indentation and blood vessels seen at the tecto-diencephalic junction in the normal animal. In brain sections of such animals, tectal histogenesis can often be seen to progress from this new junction, forwards in anterior tectum and backwards, as normally[10], in the posterior. As the recording electrode was moved successively backward from the anterior pole of the anterior tectum to the posterior pole of the posterior tectum, positions in the visual field from which electrical responses could be evoked also shifted from the anterior pole to the posterior pole. Note that, throughout, we use the terms anterior and posterior in relation to the animal's present body axis, rather than any original polarity of tissue in the embryo. Figure 3*a* shows the visuo-tectal map obtained from one such animal, a histological section of which is exhibited in Fig. 2*b*. Similar anatomy and map were observed from two animals whose tectal rudiment had been rotated at stage 37 (Fig. 3*b*). Thus the array of retinal fibres ignores the anatomical consequences of operation to produce a normal, single map with respect to the visual field and animal's body axis, by distributing itself across the two tecta. We therefore conclude that tectal tissue itself, rather than having assumed a fixed axial polarity for selective nerve connections, remains subject to overriding influences from outside the tectum until at least some 13 h after the equivalent retinal polarity is irrevocably determined[2–5].

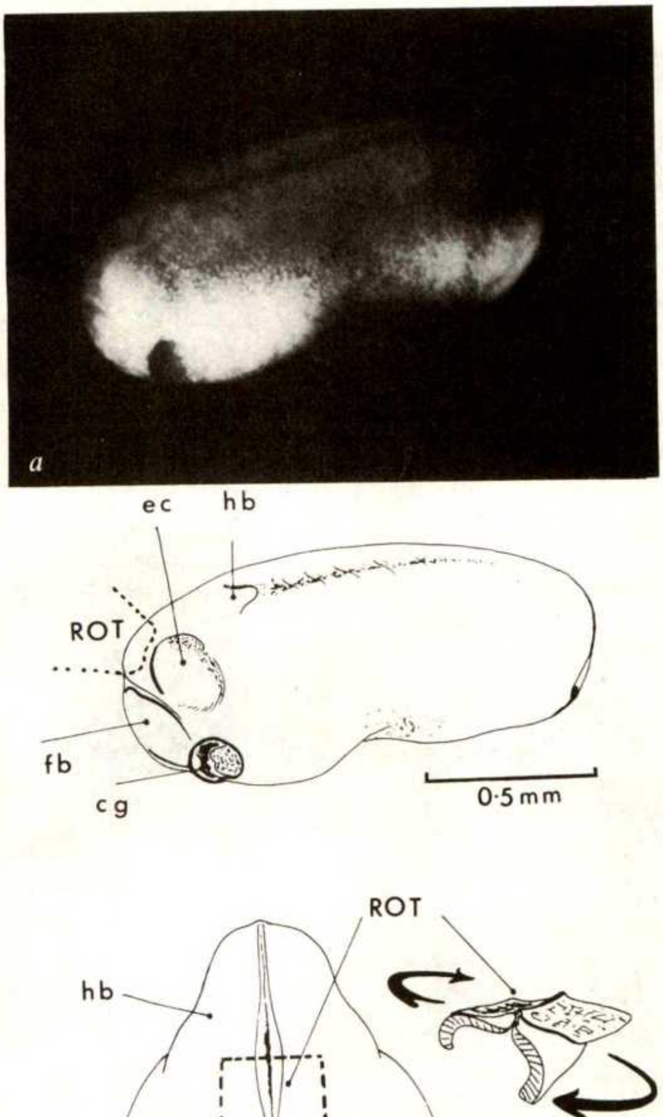

Fig. 1 Embryonic operation. *a*, Photomicrograph and *camera lucida* drawing of a stage 24 *Xenopus* embryo. Embryos were staged according to the tables of Nieuwkoop and Faber[1]. Operations were performed with sharpened tungsten needles and hair loop, in two-thirds strength Niu Twitty solution brought to *p*H 7.1 with diluted HCl. On the drawings, the heavy dashed line marks the limits of tissue reversed in a typical operation. *b*, Drawing of the head from anterior–dorsal aspect at stage 24. ec, Eyecup; hb, hind-brain; cg, cement gland; fb, forebrain vesicle; ROT, rotated piece of neural tube and overlying epidermis.

In many animals of the present category, particularly those where the new junctional groove and blood vessels were conspicuous between the anterior and posterior tecta, the path of the optic nerve was altered. Instead of entering the tectum at its anterior border[13], the optic nerve now invaded both tecta from the position of the new intervening groove. Evidence for this is threefold. First, massive responses with large receptive fields were obtained at the junction point, instead of at anterior poles of both tectum and visual field as in normal animals[10]. Second, we observed instances in which both the anterior pole of the anterior tectum and posterior pole of the posterior one were electrically silent. Finally, histological preparations of certain animals clearly show ascent of fibres between the two tecta. We therefore believe that factors normally guiding these fibres to the tectum are distinctive to the diencephalon –midbrain junction, which has been transferred posteriorly in these operations. Regardless of the trajectory of the optic nerve, incoming fibres treated the two tecta as one and distributed themselves with correct order and polarity, thereby showing that control of the direction of innervation and of progressive tectal differentiation are quite independent of the polarity of the visuo-tectal map.

Reversal of visual maps related to position of diencephalon

When the positions of cuts, at operation, were brought forward by only some 5–10 cells in the neural tube, the diencephalic structure (epithalamus, thalamus and hypothalamus) developed behind the tectum, rather than in front (Fig. 4*a* and *b*). Thus a small group of precursor cells, at stage 24, are determined as diencephalic; they form the diencephalon even when moved to a position adjacent to hind-brain precursor cells. The anterior pole of the tectum in such animals was joined directly to the telencephalon, whereas normally the diencephalon is interposed between these two structures (compare Fig. 4*a* and *b*). A typical visuo-tectal map obtained from one such animal is shown in Fig. 4*c*. In contrast to a normal map, the anterior visual field was now represented in the posterior pole of the tectum, and the posterior in the anterior pole; the visuo-tectal map was completely reversed.

The medio–lateral polarity, unlike the anterior–posterior one, was not reversed. As in normal animals, fibres stemming from the ventral part of the retina (namely, those subserving the superior visual field, as the lens inverts the image) positioned themselves more medially than those from the dorsal retina. Because the original operation was symmetrical about the animal's midline, we do not expect, and indeed have never found, any reversal in the medio–lateral axis of maps.

Following the version of the operation just discussed, 13 animals developed two separate tecta on each side, separated by the diencephalon (Fig. 5*a*). This presumably occurred because of inevitable variability in the operation, whereby in these cases some tectal precursor tissue had been left unrotated posteriorly.

A parasagittal section of such an animal shows clearly that the infundaibular recess and hypothalamic nuclei lie directly below the level at which the two tecta join, and the cellular mass constituting the dorsal and ventral thalamic nuclei is interposed between the tecta (Fig. 5*a*). In trans-

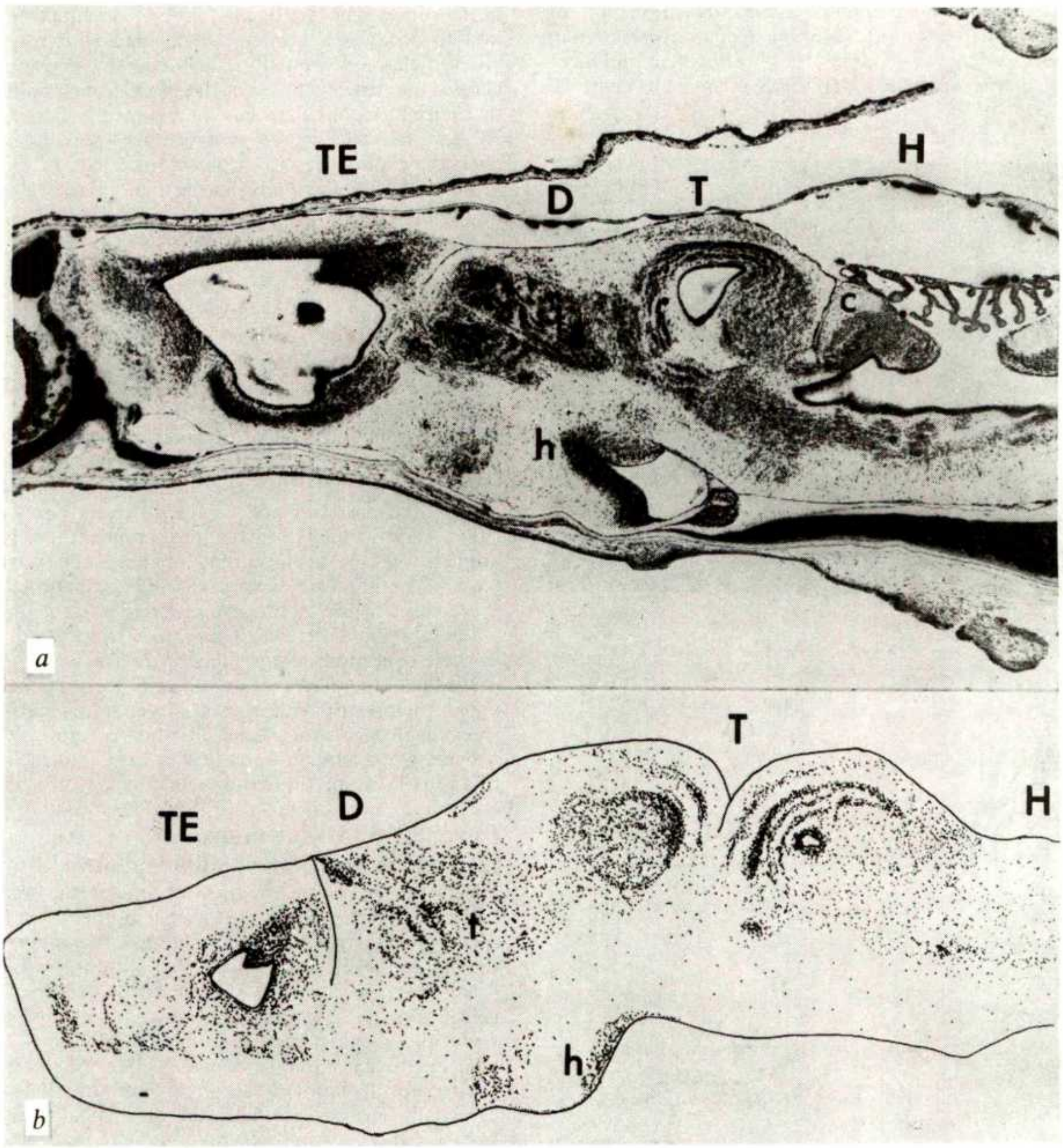

Fig. 2 Parasagittal sections of brains from (*a*) normal and (*b*) experimental tadpoles at stage 66. To make the rearranged structures more apparent, we have selected parasagittal sections throughout, although detailed anatomy is best followed in serial transverse sections. Because the experimental (*b*) animal was one also treated for ^{3}H-autoradiography, a *camera lucida* drawing is given since the visual appearance of the tectum is not comparable with the control (*a*) or with subsequent sections shown. TE, Telencephalon; D, diencephalon; T, tectum; H, hind-brain; h, hypothalamic nuclei; t, thalamus; c, cerebellum.

verse sections, not shown here, different diencephalic nuclei, including those of the epithalamus, can be identified more clearly. The pattern of visuo-tectal map in these animals showed that a complete visual field was represented in each tectum. The polarity of the map in the anterior tectum was reversed, whereas that of the posterior tectum was normal (Fig. 5*b*). Thus, the population of incoming fibres distributed themselves across the two tecta, those originating from the posterior part of the retina occupying, as in the normal brain, that tectal tissue adjacent to diencephalon.

In several animals, an extra diencephalon developed anterior to the tecta, in addition to the one between the two tecta. Embryologically, such an anomaly would be expected if some diencephalic cells were left *in situ* and some were translocated, and both groups then regulated to give complete structures. The anterior tectum, situated between the two diencephalons, showed two superimposed maps with opposing polarity, as illustrated in Fig. 6. When the recording electrode was at the most anterior pole of this tectum, responses could be elicited both from the far anterior and far posterior parts of the visual field. Similar responses could be obtained from the posterior pole of this tectum. Two ordered series of symmetrical pairs of responses were then obtained as the electrode positions advanced along the intervening tectum. Such overlapping projections of opposing polarity indicate that each diencephalon can exert its own polarising influence on the intervening tectum, independently of the other.

Implications for selective nerve connections

Several inferences can be made from our study about the mechanisms governing formation of selective nerve connections. The most general conclusion we can draw from our findings, without recourse to any specific notions previously advanced, is that polarity for selective nerve connections within tissues remains labile long after that for the anatomical pattern of the brain as a whole is fixed. The possibility remains, however, that the same positional

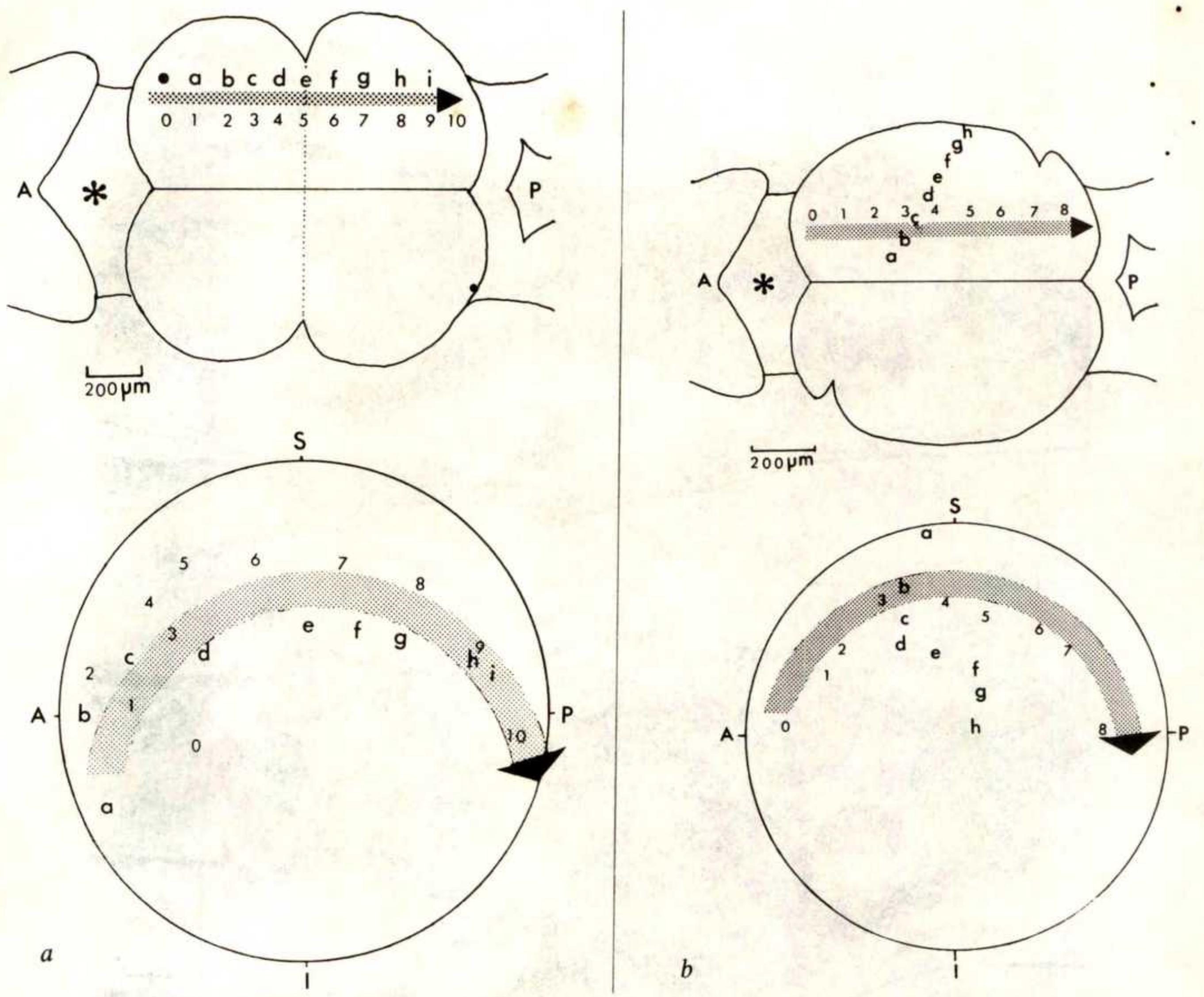

Fig. 3 Normal visual map after rotation of *a*, neural tube at stage 24 and *b*, tectal rudiment at stage 37. The map (*a*) was obtained from the animal whose histological section is displayed in Fig. 2*b*. All animals discussed in this paper were mapped between stages 62 and 66. Many rows of electrode positions were tested, but for the sake of simplicity, we show in this and following figures the results for only one or two rows. Positions in particular rows are either numbered or lettered consecutively, and positions in the visual field from which responses were obtained are then shown. Overall polarity of rows is shown for quick inspection by broad shaded arrows. Asterisks indicate the location of the diencephalic structure. A, Anterior; P, posterior; S, superior; I, inferior.

signal[9] is involved first in morphological determination and subsequently in establishing axial reference for selective nerve connections. The nature of such signals and of the cellular responses to them remain unknown. Nevertheless, our findings provide a basis for experimental attack on these problems in developing nervous systems.

Our results also suggest that a group of cells other than the tectal precursors can organise axial information within the developing tectum for incoming optic nerve fibres. These cells appear to be some or all of those which are determined to form the diencephalic structure itself. This conclusion is strengthened by results of the first category of animals (Figs 2, 3 and 4). Polarity of visuo-tectal maps in these animals was normal, although tissue normally situated at the very junction of diencephalon and tectum had been transferred posteriorly. The diencephalon thus may act in a way quite analogous to an embryonic organiser, in that each of them controls an axial polarity in surrounding tissue. In the case of the diencephalon, the polarity is that for the retino-tectal connections. Classical organisers, on the other hand, control anatomical patterns of cellular differentiation in surrounding embryonic tissues[9,14,15]. It is relevant to note here that the organiser for the axial pattern of the whole body during amphibian development is a small group of cells at the dorsal lip of the blastopore, which later form the anterior mesoderm[8,14,15]. This mesoderm then induces in overlying ectoderm the formation of the forebrain rudiment, of which the diencephalon is the central part. An obvious test for the hypothesis of diencephalic organiser is to transplant a presumptive diencephalon alone as a graft behind the presumptive tectum of the host. Our preliminary results from a series of such animals are conforming with our expectation.

Several models have been proposed for the formation of ordered retino-tectal connections during development[8,16–18]. Before trying to evaluate these models, one has to ask a fundamental question: how much information is encoded in each tectal cell? Maximum information is assigned to each tectal cell if each possesses a uniquely labelled positional code[6]. On the other hand, the minimum possible information is assigned to the tectum if there is only one reference point within or outside it, to align properly the projection of incoming fibres, and no positional memory is encoded in individual tectal cells[16].

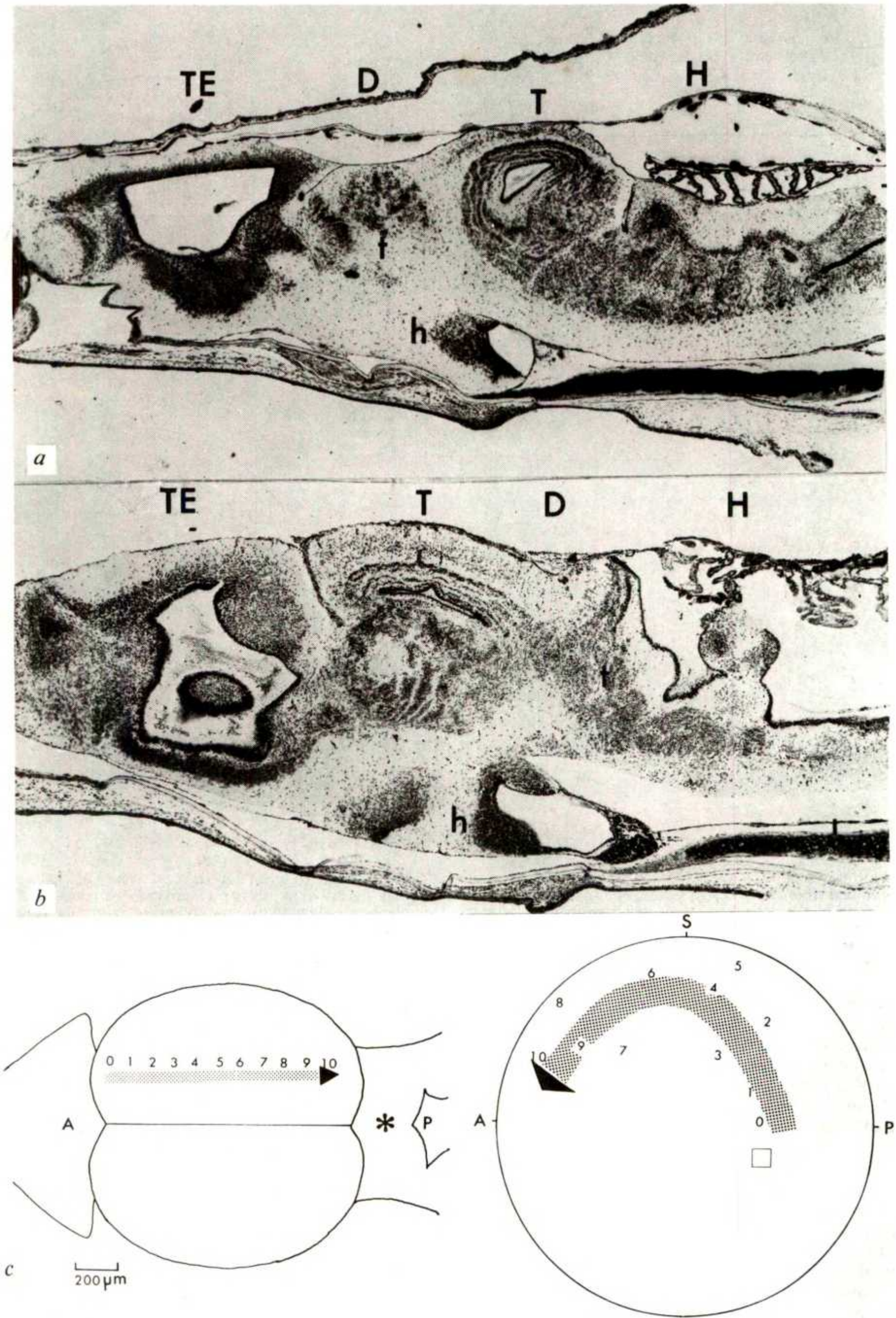

Fig. 4 Reversed visual map with diencephalon situated behind the tectum. Comparable parasagittal sections of *a*, normal and *b*, experimental animal (operated at stage 24). *c*, Reversed visuo-tectal map across the single tectum. The map and the histological section (*b*) were obtained from the same animal. From the electrode positions 2, 3, 4, 5, 6 and 8, responses could also be elicited by stimulating the visual field marked by the square. We can offer no plausible explanation for this anomaly. See Figs 2 and 3 for legend and abbreviations.

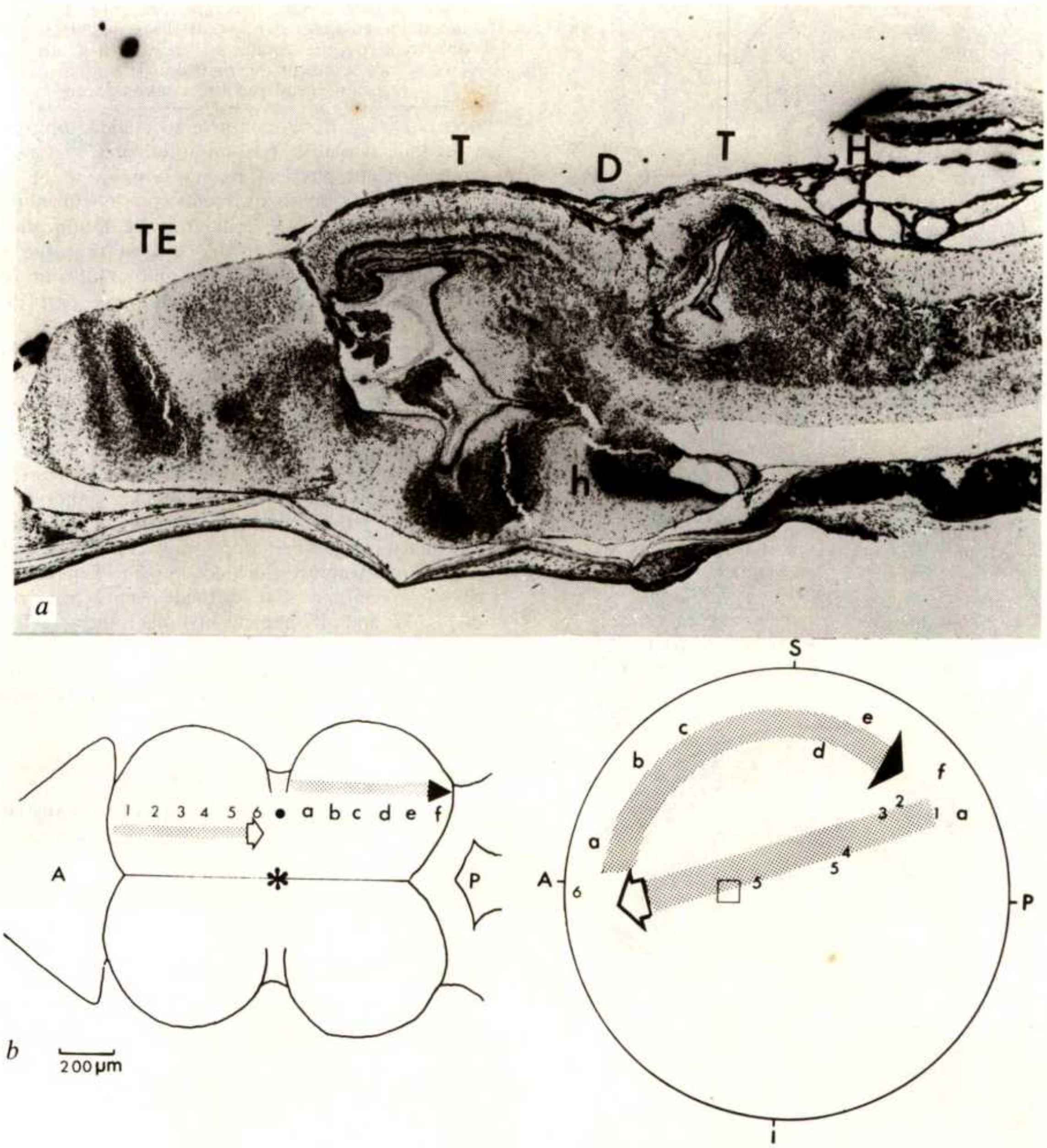

Fig. 5 Transposition of the diencephalon to lie between anterior and posterior tecta (operation at stage 24). *a*, Parasagittal section. *b*, Visuo-tectal map showing complete reversal for the anterior tectum and normal representation in the posterior tectum. The map and the histological section were obtained from the same animal. From the electrode positions 2, 4 and 6, responses could also be elicited by stimulating the visual field indicated by the square. See Figs 2 and 3 for legend and abbreviations.

Our present results show that positional labelling within tectal cells, if such ever occurs, can be overridden at least until stage 37 (Fig. 3*b*). Since functioning synapses are present by stage 45 in normal tadpoles[19], any positional labels having significance in ordering connections must have been acquired within the intervening period. Because the tectal rudiment at such stages is fragile and sticky, we have not succeeded in eliminating this possibility. The observation of dual projection patterns with opposed polarity (Fig. 6), across the same tissue, nevertheless makes it difficult to suppose that tectal cells possess unique labels before optic innervation.

Our results are equally consistent with a theory which requires no positional memory in tectal cells. If we assume that, in addition to a reference point in the nervous system, there is a mechanism whereby optic nerve fibres can preserve, on the tectum, the spatial contiguity of their origin in the retina, a wide range of experimental data including ours can be accounted for. The idea that mechanisms other than graded chemical labelling may be used by the nervous system in maintaining spatial contiguity, has been advanced previously. Lettvin's notion (cited in Chung[18]), for instance, is based on the fact that dendritic trees of retinal ganglion cells arborize extensively and interdigitate with neighbouring ones. Two fibres arising from adjoining retinal ganglion cells are, therefore, likely to exhibit a temporal contiguity in their discharge patterns, and such a coincidence in firing can, in principle at least, be used for maintaining spatial relations between the fibres. An alternative notion, advanced by Goodwin and Cohen[20], makes use of a signalling system arising from oscillatory metabolic processes in assigning positional codes to retinal and tectal elements, which then match up. In both of these models, each tectal element need not be uniquely labelled in order to establish ordered connections with retinal fibres. An underlying assumption in these

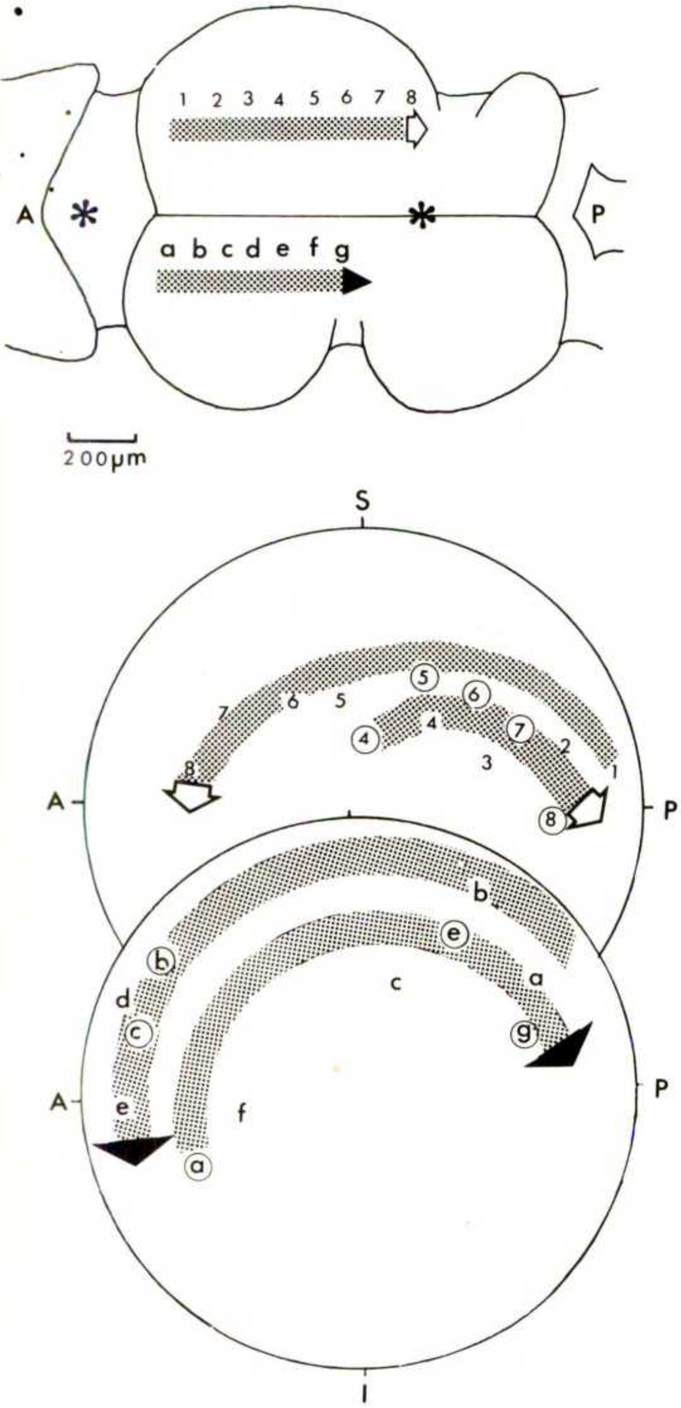

Fig. 6 Visuo-tectal maps obtained from tecta lying between 2 sets of diencephalic structure (operation at stage 24). Two superimposed ordered maps of the visual field with opposed polarity across the tectum, are seen on each side of the brain. Two circles represent the right and left visual fields. See text. For legend and abbreviations, see Fig. 3.

models is that fibres compete to achieve optimal matching with the available post-synaptic sites[17,18]. Whether such models reflect physical reality remains to be investigated.

Experiments involving tectal rotation in adult *Xenopus*[21] and goldfish[22,23] as well as transplantation studies on the *Rana catesbiana* tectum[24,25], suggest that the situation in the adult brain may differ from that during development. For instance, when tissues from one part of the adult tectum were interchanged with those from another part, they appeared to retain memory of their previous position in 13 cases[25], whereas no such information was retained in 14 other cases[24]. The conditions in which these two occur must first be ascertained.

A final evaluation of the two classes of theory we have outlined must, however, await further investigations. We propose that before tectal cells are innervated by optic nerve fibres, they possess no positional memory, and axial information for incoming optic fibres originates from a polarising structure located in the diencephalon. It can be shown either that critical events occurring between stages 37 and 45 irrevocably label tectal cells, or that a properly aligned map can be established in a developing tectum *in vitro* without diencephalic tissue, then our theory, in its simplest form, will have proved to be wrong.

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letters to nature

Radio galaxies and local quasars

A RECENT discovery regarding Centaurus A (NGC5128)[1] may have an important bearing on the very local quasar model (see for example, refs 2 to 5). These observers, using the new 4-m telescope at Cerro Tololo Observatory, have obtained remarkable photographs showing visible filaments extending away radially from the galactic centre and blue starlike images never before seen near the Galaxy. These starlike objects might have been called quasi-stellar had this term not taken on additional connotations since 1963, and this name may indeed be the proper one.

Such blue stars, presumably ejected from the galactic centre, are just what the very local quasar model predicts. They have been found in the area of the north-east radio lobe of Cen A at a distance of perhaps 80,000 light yr from the centre of that galaxy. If they have brightness similar to Type O stars, roughly 10,000 times brighter than the

Sun, they would appear as twelfth-magnitude stars as observed from the centre of NGC5128. Thus, to hypothetical astronomers in that galaxy, they would appear a little brighter than the quasar 3C273 as seen from Earth. These starlike objects near NGC5128 would then be of magnitude 23 or 24 as observed here, near the limit of observation.

If these blue stars are radio sources, as seems quite possible because of their location in the radio lobe, they could be producing radio emission by the same mechanism postulated for very local quasars[2–5], that is, by relativistic motion through the gas and magnetic fields near the galaxy which ejected them. This would produce synchrotön radio emission in great strength and over a long time, drawing its power from the kinetic energy of the relativistically-moving star. It will be of considerable interest for radio astronomers to determine whether these blue objects are indeed radio sources.

In any case, the radio emission is coming from their vicinity, and the radio output and blueness give them some of the properties expected of local quasars—local to the galaxy NGC5128. If these objects are moving with relativistic speeds their proper motions would be ~ 0.01″ yr^{-1}, and might just be observable over a number of years. Spectroscopy to determine the presence of a redshift or a blueshift would be very difficult, if not impossible, at this magnitude—near the limit of observation. Fluctuations in the optical and radio brightness would, however, be expected if these objects are indeed quasars local to Cen A, and should be looked for.

The energy required by the powerful radio emission from galaxies such as Cyg A and Cen A, which have strong emission from regions hundreds of thousands of light-years from the galactic centre, has been as much of a problem to explain as the energy source of quasars if cosmologically distant. Thus it may well be necessary to postulate the existence of very local quasars, massive objects thrown out (whether before or after condensation) from a gravitational collapse at the centre of a galaxy, in order to account for the puzzling properties of radio galaxies[2–5]. To inhabitants of those galaxies they would look much the same as quasars look to us, so that the explanation may well be the same in both cases.

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Λ-type doubling in SiH molecule

In recent years there has been much interest in the detection of the radio-frequency spectra of interstellar molecules. The observed spectra of the molecules OH and CH corresponded to transitions between the Λ-doubling components of the lower rotational levels. While experimental determinations proved very difficult, theoretical calculations on the Λ doubling in the lowest rotational level of CH have been notably successful[1,2]. If the radioastronomical observation[3] is corrected for the epicentre and the calculated value for centrifugal distortion, then the agreement of theory and experiment for the radio-astronomical frequency is within 2 MHz in over 3,000 MHz.

SiH is similarly of interest as a probable interstellar species, but in this case the only available prediction for the radio-astronomical frequency band must be made by extrapolating Λ-doubling from high rotational levels populated in laboratory experiments to low rotational levels.[4]

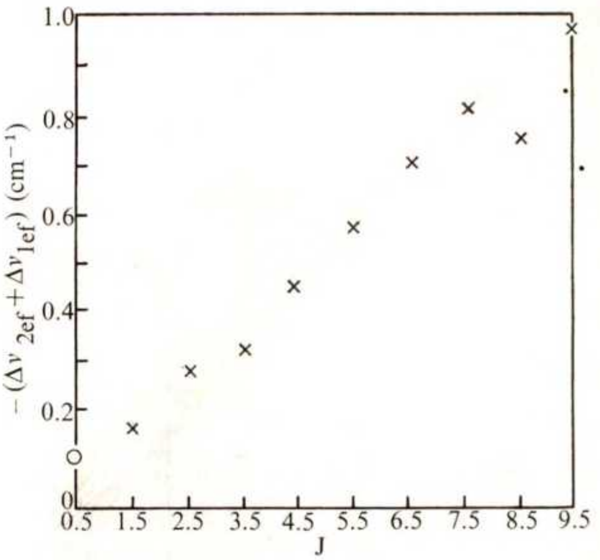

Fig. 1 Extrapolated experimental Λ-doublet splittings and the calculated value, experimental values of $-(\Delta\nu_{2ef}+\Delta\nu_{1ef})$ come from ref. 10, ×, experimental points; ○, theoretical calculation for $J=\frac{1}{2}$.

Calculations are more difficult for SiH than for the similar CH species, since less is known about the perturbing excited states, and extra complications are introduced by the presence of a second row atom[5]. Nonetheless, predictions can be made if the approximations introduced by Van Vleck[6] are used. To second order the splitting of energy levels of a $^2\Pi$ state may be expressed in terms of two parameters p and q, and the splitting in the lowest rotational level of a $^2\Pi_{\frac{1}{2}}$ state is given by $(\frac{1}{2}p+q)$, where:

$$p=4\sum\frac{\langle{}^2\Pi|H_{\mathrm{SO}}|{}^2\Sigma\rangle\langle{}^2\Sigma|B(L^{+}+L^{-})|{}^2\Pi\rangle}{E_{\Pi}-\mathrm{E}_{\Sigma}}$$

$$q=2\sum\frac{|\langle{}^2\Sigma|B(L^{+}+L^{-})|{}^2\Pi\rangle|^2}{E_{\Pi}-E_{\Sigma}}$$

The summations extend over all vibrational levels of all $^2\Sigma$ states, with contributions from $^2\Sigma^-$ states being taken as negative. Matrix elements are taken over both electronic and vibrational wave functions; H_{SO} is the spin-orbit coupling operator and B is the operator $h/8\pi\ \mu r^2$.

Table 1 Λ-type doubling in the X $^2\Pi_{\frac{1}{2}}$ ($v=0$, J $=\frac{1}{2}$) state of SiH

	p(cm^{-1})	q(cm^{-1})
Contribution from B $^2\Sigma^+$	0.02867	−0.00119
Contribution from $^2\Sigma^-$	0.05393	0.01273
Total contribution	0.08260	0.01154
Extrapolated experimental doubling	2,940 ±300 MHz	
Calculated doubling	3,168 MHz	

Attention is restricted to the influence of the B $^2\Sigma^+$ state and an unobserved $^2\Sigma^-$ state. Electronic wave functions for the X $^2\Pi$ state and the $^2\Sigma$ states were obtained using the ALCHEMY *ab initio* wave function computer program[7], including configuration interactions, using six configurations for the X $^2\Pi$ state, eight for the B $^2\Sigma^+$ state and five for the $^2\Sigma^-$ state.

Vibrational wave functions were obtained from the computed multiconfigurational potential curves for the X $^2\Pi$ and B $^2\Sigma^+$ states, but the $^2\Sigma^-$ state is found to be repulsive. For this latter state vibrational wave functions were obtained using the box normalisation technique of Gordan[8] and Czarny *et al.*[9] and the contributions to p and q have to be integrated over the energy of the state.

The various contributions to p and q and the predicted doubling of the lowest level of SiH $^2\Pi_{\frac{1}{2}}$ are given in Table 1. The figure shows the theoretical prediction compared with the experimental extrapolations. The agreement is close enough to provide some encouragement for a search for the molecule in interstellar space to be conducted, using available receivers.

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Haze in the stratosphere

On September 1, 1975 I flew as a passenger on Concorde flight OF269 from Heathrow to Gander taking off shortly before 0730, arriving at Gander shortly before 1000 (London time). The return journey on flight OF277 started an hour later and arrived at Heathrow shortly before 1400. Over the Atlantic we climbed gradually from ~ 50,000 feet to ~ 56,000 feet.

I watched carefully for visual evidence of the tropopause throughout the flights. During the descent to Gander cirrus clouds extended up to 44,000 feet. There was occasionally slight clear air turbulence, sometimes regular enough to indicate a billow wavelength of ~ 120 m, at many different heights in the stratosphere, but most remarkable of all was the extent of haze layers.

The distant horizon was the cirrus cloud top which was variously at heights between 35,000 and 44,000 feet. Above that the sky had a whitish glow, and as we ascended, several discrete layers, in which the haze was denser, were revealed as we came to the top or bottom of the layer. When the sun was low, on the outward flight, they were brown coloured in the north-west; when the sun was high on the return flight at the eastern end, they glowed white in the south-east. I had a forward starboard window. The highest layers were still above the aircraft at 56,000 feet, and the sky glowed with a paler blue than on occasions when I have observed the colour of the stratosphere from jet aircraft, for example Comets, at ~ 35,000 feet. On this occasion the weather ship radiosondes indicated a very obvious tropopause at ~ 39,000 feet but it certainly did not stand out visually.

The haze extended all the way across the Atlantic, and its extent is very significant. We tend to think of haze as a cooling agent for the air, because that is what it is in normal low altitude pollution situations; but on this occasion it was a warming agent, being probably below the radiation equilibrium temperature between the Earth and outer space. Thus the presence of haze facilitates the ascent of air without adiabatic cooling in the stratosphere, and on this occasion could well have been associated with the exceptionally large descent taking place above hurricane Donna further south in the Atlantic.

The importance of this observation is that it makes nonsense of the assumption, implicit in almost all theories concerning the consequences of putting pollution into the stratosphere, that practically everything that can, in the usual rainmaking processes, be rained out as air ascends into the stratosphere, actually is rained out and thus removed. This includes NO_x, sulphur compounds, such as SO_3, and ammonium sulphate, and a large range of organic compounds of which the most important, in today's context of ozone depletion scares, is methyl chloride.

It is probably not necessary to look further than the normal natural interchange mechanisms across the tropopause for the greatest supply of all these substances to the stratosphere, and it is easy to stipulate that mother-of-pearl clouds are formed on layers of sulphate haze at heights around 80,000 feet. The implications of these observations make nonsense of the ozone depletion theories which depend absolutely on a degree of stratospheric purity that is quite unrealistic. Furthermore the ascent of such a vast extent of air on an occasion such as this makes nonsense of the supposition that the vertical transport mechanisms in the stratosphere can be correctly represented by diffusion mechanics in which all ascending chemical species mix and react chemically with all descending chemical species. Ozone depletion theories are based completely on diffusion models.

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Continuous plankton records show fluctuations in larval fish abundance during 1948–72

The continuous plankton recorder (CPR) survey[1] has sampled the plankton at a depth of 10 m around the British Isles consistently since 1948. Analysis of this material has revealed a number of trends[2,3] in the distribution, abundance and timing of seasonal cycles of zooplankton and phytoplankton. This report discusses similar changes in the distribution and abundance of planktonic fish larvae.

Counts of fish larvae from selected recorder samples are allocated to statistical rectangles of 1° latitude by 2° longitude, and the mean number of fish larvae per recorder sample per month is calculated for each rectangle. These means are then used for the analysis of variability in distribution and abundance.

In the North Sea, at a depth of 10 m, there has been a decline in abundance of some larvae (for example *Odontogadus merlangus* (whiting), *Trisopterus esmarkii* (Norway pout), *Limanda limanda* (dab) and *Pleuronectes platessa* (plaice), and for some species (for example *O. merlangus*, *P. platessa and Ammodytes* spp. (sand eels)), a shortening of the season during which the larvae have been caught, and a delay in the time of the seasonal maximum abundance. Examples of these changes are given in Fig. 1, which shows monthly and annual fluctuations in the abundance of larvae of *O. merlangus* and *P. platessa* from recorder sampling in the North Sea. The larvae of both species declined in abundance over the period; the downward trend of *O. merlangus* started in 1964, whereas for *P. platessa* there has been a general trend over the whole 25-yr period. In both species the time when most larvae were sampled has shifted to later in the season and the season itself has shortened. These patterns bear a close resemblance to those found for the zooplankton and phytoplankton in the CPR survey; over the same period the total numbers of copepods and the zooplankton biomass in the North Sea have declined[2], the duration of the

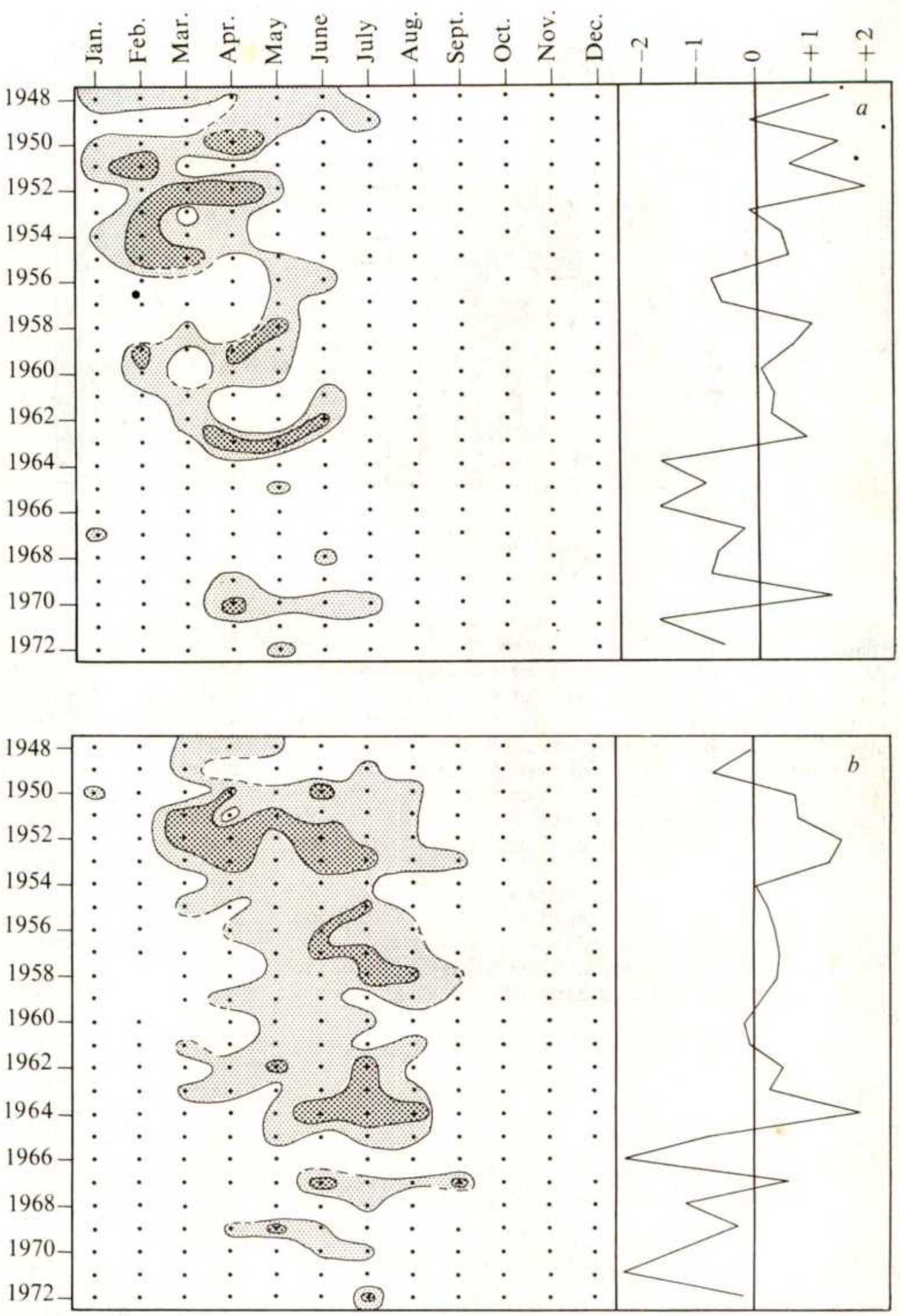

Fig. 1 Monthly fluctuations in abundance of: *a*, *Pleuronectes platessa* and *b*, *Odontogadus merlangus* from 1948 to 1972, obtained from CPR sampling in the North Sea. Contour levels as mean numbers of fish per recorder sample are 0 and 0.05 for both diagrams; missing dots represent months with inadequate sampling. The right hand section of each diagram shows the fluctuations in annual abundance, standardised about a zero mean and plotted as standard deviation units. Annual abundance indices were obtained by summing monthly means.

zooplankton season has become progressively shorter[2], and the spring bloom of phytoplankton has become progressively later[2].

Similar changes have been recognised in CPR sampling of young fish in the English Channel and north-eastern Atlantic; charts showing the distribution of a common mid-water fish, *Stomias boa ferox* (dragonfish), for two periods are illustrated in Fig. 2. From 1948 to 1967 larvae were found only over deep water beyond the continental shelf, whereas from 1968 to 1972, the northern limit of distribution was much further south, and larvae were found over the shelf extending into the English Channel. These larvae had not hatched recently, so that their occurrence in the Channel was probably the result of drift from their spawning areas near the continental slope. Such an incursion of larvae into the Channel, observed since 1968, supports the hypothesis of an increased easterly flow of water through the Channel since about 1965, as proposed by Russell *et al.*[4].

A southerly shift in the distribution of other fish larvae (like that of *Stomias* after 1967) is apparent from the CPR survey (for example *Melanogrammus aeglefinus* (haddock), *Micromesistius poutassou* (blue whiting)[5,6], *Scomber scombrus* (mackerel), and *Hippoglossoides platesssoides* (long rough dab)), and similar changes in the geographical distributions of fish[4,7] and other marine animals[4,8], have been reported indicating an increased boreal influence from about 1965.

The seasonal period during which most of the *Stomias* larvae are found in the Western Approaches has shifted from March and April (between 1948 and 1961) to April, May and June (from 1968 to 1972). Since about 1965 there have been indications of a coincident shift in the main season of abundance of pilchard eggs in the Channel to later in the year[4].

These changes in timing, abundance and distribution of fish larvae in the CPR survey add to the increasing evidence[4,7,12] that many, perhaps most, pelagic organisms underwent a marked

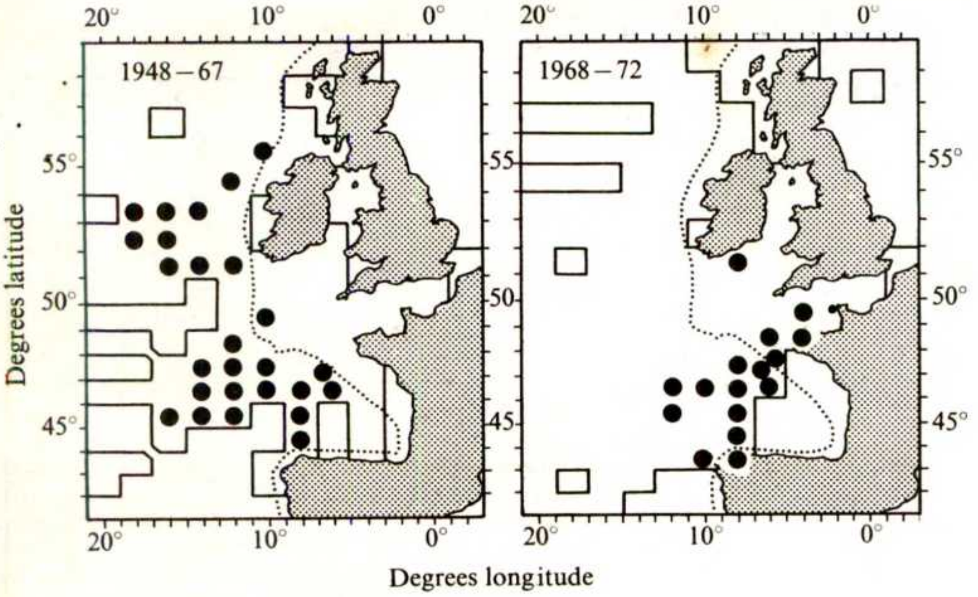

Fig. 2 The distribution of the larvae of *Stomias boa ferox*. Black dots indicate statistical rectangles in which larvae were found. The sampled area, from February to October, inclusive, is outlined, and the 200-m contour shown by a dotted line.

change in about 1965–67, in the waters of the north-western Atlantic and North Sea. Such a coherent and widespread pattern of change seems likely to have had its origin in climatic events[9,10]. It is conceivable that climatic change may have affected the distribution of the adult fish, here reflected in the larval distribution, but it is also possible that the changes in the fish larvae have been caused by corresponding changes in the primary and secondary trophic levels, arising from the dependence of survival of young fish on the availability of prey organisms[11].

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Structure of graphite by neutron diffraction

THE structure of graphite[1] consists of layers of linked hexagons of carbon atoms. The layers are stacked in a $\cdots$ ABAB $\cdots$ sequence so that half the atoms in a layer are directly above and below carbon atoms in the adjoining layers, and half are directly above and below centres of the hexagons. The hexagonal unit cell contains atoms at the (0, 0, 1/4), (1/3, 2/3, 1/4), (0, 0,–1/4) and (2/3, 1/3,–1/4) positions. Modifications to this structure have been proposed by several workers. Lukesh[2] reported diffraction patterns indicating a lower symmetry, and Pauling[3] has proposed an orthorhombic structure in which the interconnected six-membered rings are distorted so that two-thirds of the bonds are longer. Pauling based his model on the high compressibility of the basal planes, and considerations of stacking bonds in the layered structure. Ergun[4] suggested that data on pyrolitic graphite indicated an apparent quinoid structure in the graphite layers, but errors in his analysis have been reported[5]. X-ray and neutron diffraction studies of graphite were undertaken in our laboratory to study the details of the electron charge density and atomic motion in the graphite structure. The results of the neutron diffraction work show that all in plane carbon–carbon distances have the same value 1.422 ± 0.001 Å.

X-ray diffraction photographs of almost seventy small, natural single crystals of graphite indicated that two or three were of exceptional quality. The largest was a six-sided plate 0.2 mm thick, approximately 2 mm long and 1.4 mm across. This crystal was glued to a glass fibre, so that the axis of the fibre was parallel to the basal planes of the sample. Measurements were made on the full-circle automatic diffractometer at station H6S3 of the Brookhaven HFBR. A Ge (220) reflection was used to make the beam monochromatic, with wavelength of 1.02041 Å. Vertical in-pile soller slits and horizontal soller slits between the monochromator and sample were used. A $\frac{1}{2}'' \times \frac{1}{2}''$ receiving slit, 16″ from the sample and in front of the detector, was used. Twenty-seven reflections equally distributed over the diffracting sphere were centred to obtain accurate cell dimensions. A least-squares refinement of the angular positions of these reflections resulted in the unit cell dimensions and angles shown in Table 1. Assuming a triclinic cell, the a and b axes were equal to within σ (estimated standard deviation) and the angles between the unit cell vectors were 90°, 90°, and 120°, within 1–2σ. Refinement assuming a hexagonal cell led to the lattice parameters also shown in Table 1.

Intensity data were obtained for 339 reflections, including all symmetry-related reflections within the 0.75-Å diffracting sphere. Step scans through each reflection were made in the

Table 1 Unit cell refinement

Triclinic cell	Hexagonal cell
$a = 2.464 \pm 0.002$ Å	$a = b = 2.464 \pm 0.002$ Å
$b = 2.466 \pm 0.002$ Å	$c = 6.711 \pm 0.004$ Å
$c = 6.711 \pm 0.004$ Å	
$\cos\alpha = 0.0003 \pm 0.0004$	
$\cos\beta = 0.0005 \pm 0.0003$	
$\cos\gamma = -0.5005 \pm 0.0003$	

Table 2 Graphite structure refinements

		$P\bar{1}$ full sphere no restrictions	$P\bar{1}$ full sphere $z = 1/4$	$P2_1/m$ 1/4 sphere $z = 1/4, y = 1-x$	$P6_3/mmc$ 1/24 sphere (0, 0, 1/4), (1/3, 2/3, 1/4)
C_1	x	0.0004 ± 0.0006	0	0	0
	y	0.0001 ± 0.0005	0	0	0
	z	0.2500 ± 0.0002	1/4	1/4	1/4
	U_{11}	0.0031 Å^2	0.0031 Å^2	0.0031 Å^2	0.0031 Å^2
	U_{33}	0.017 ± 0.001 Å^2	0.017 ± 0.001 Å^2	0.016 ± 0.001 Å^2	0.016 ± 0.002 Å^2
C_2	x	0.3326 ± 0.0005	0.3325 ± 0.0004	0.3331 ± 0.0004	1/3
	y	0.6663 ± 0.0005	0.6664 ± 0.0004	0.6669	2/3
	z	0.2501 ± 0.0002	1/4	1/4	1/4
	U_{11}	0.0031 Å^2	0.0031 Å^2	0.0031 Å^2	0.0031 Å^2
	U_{33}	0.017 ± 0.001 Å^2	0.017 ± 0.001 Å^2	0.017 ± 0.001 Å^2	0.017 ± 0.002 Å^2
R		4.6%	4.6%	4.9%	4.2%

θ–2θ mode with a scan width of 2θ of 4.5°–6.0° and a step width of 0.06°. Of the 339 reflections measured, 52 would have zero intensity if the classical structure is correct. Of these 52 intensities, 38 were less than σ, 48 were less than 2σ and four could not be reduced because of uneven backgrounds or fluctuations. The standard deviations were based on counting stastistics. No attempt was made to measure these reflections to a high degree of accuracy, and they were not used in the succeeding refinements. Examination of the intensity data indicated extinction effects were small and these corrections were neglected.

Thermal parameters of the form $\exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}l^2-2hk\beta_{12}-2hl\beta_{13}-2kl\beta_{23})$ were used for C_1 and C_2. Initial refinements resulted in values of β_{11}, β_{22}, β_{12}, β_{13} and β_{23} that were smaller than estimated standard deviations for these parameters. Refinements setting these parameters equal to zero, and equal to values derived from other measurements of the mean square amplitude of vibration in the basal plane were carried out. No significant effects on the positional parameters were observed. The results of the refinements (Table 2) were obtained by assuming mean square amplitude of vibration of 0.0031 Å^2 in the basal plane, based on neutron measurements by Ludsteck[6].

Four full-matrix least-square refinements were done with varying restrictions on the structural parameters. In the first, a centre of symmetry was taken at the origin and no restriction was put on the positions of the two independent atoms in the unit cell, C_1 and C_2. The results of this refinement in space group $P\bar{1}$ indicated that the atoms in the basal planes are planar to within ±0.001 Å, and the classical structure of graphite is consistent to within 2σ. In the second refinement, it was assumed that the basal planes were truly planar, enabling one to fix the position of C_1 and refine only the x, y coordinates of C_2. The results of this refinement were not significantly different from the first. Ergun's proposed structure[4] restricted the position of atom C_2 so that the x, y coordinates were related by $y = 1-x$. The intensity data were averaged to a quarter sphere assuming

$$I(hkl) = I(\bar{h}\bar{k}\bar{l}) = I(hk\bar{l}) = I(\bar{h}\bar{k}l).$$

This refinement in space group $P2_1/m$ provided further confirmation of the classical structure of graphite. If the Bernal structure[1] is assumed, only the thermal parameters are variables in the refinement. This refinement was done averaging the data to 1/24 of the sphere. Results of all four refinements are shown in Table 2.

The thermal parameters for C_1 and C_2 are not significantly different. The mean square amplitude of vibration perpendicular to the basal plane for C_2, which has voids above and below it, is nominally higher than that of C_1. These mean square amplitudes of vibration correspond to Debye temperatures of 490±40 K and 470±40 K for C_1 and C_2 respectively. These compare with Ludsteck's determination of the average graphite Debye temperature for out-of-plane vibrations of 670^{+100}_{-70} K (ref 6).

No indication of the proposed modifications of the graphite structure can be found in our data. Pauling predicted that two-thirds of the carbon–carbon bond distances may be 1.448 Å and one third 1.366 Å. Comparable figures for the Ergun modification are 1.455 Å and 1.355 Å. Our results show that all in-plane carbon–carbon bond distances are 1.422±0.001 Å. It should be pointed out that this distance is averaged over time and over a large number of unit cells by the diffraction process.

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Photo-orientation of anisotropically absorbing molecules

THE action of plane-polarised light on anisotropically absorbing molecules produces an induced anisotropy in an ensemble of molecules, which can be detected by double refringence, dichroism and other optical phenomena. The phenomenon was first observed[1] on dye molecules in liquid[2], although its nature and mechanism by which it operates remain unexplained—neither can they be explained by the optical Kerr effect[2].

Analysis of existing experimental material[1,2] shows that there is a new kind of nonlinear optical phenomenon which takes place only upon the interaction of plane-polarised light with anisotropically absorbing molecules and which is resonant. Therefore, it can be observed at weak intensities using non-laser sources.

We suggest a classical model for an assembly of damped linear oscillators at resonance in a plane-polarised electric harmonic field. Each oscillator behaves according to the equation

$$m\ddot{x}+2mr\dot{x}+mf^2x = eE\cos\theta\cos ft \qquad (1)$$

where m and e are the electron mass and charge, respectively, r is the damping coefficient, E is the amplitude of the electric field in an incident light beam, θ is the angle between the direction of the applied force and the axis of the oscillator, and f is the resonance frequency. The total energy of the oscillator is:

$$W(\theta) = (eE\cos\theta)^2/8mr^2 \qquad (2)$$

The dependence on the angle θ leads to the appearance of a moment of force, $-(\mathrm{d}W/\mathrm{d}\theta)$, which tends to orient the oscillator perpendicularly to the direction of the electric field in a light beam. The anisotropy of the ensemble of mutually non-interacting oscillators at a given temperature, T, is subjected to the Boltzmann distribution

$$P(\theta) = C\exp(-W(\theta)/kT) = C\exp(-b\cos^2\theta) \qquad (3)$$

where $b = (eE)^2/8mr^2kT$. Under plane-polarised resonance illumination the angular distribution of the oscillators becomes non-random. This photo-induced anisotropy causes several nonlinear optical effects: deviation from Beer–Lambert law for plane-polarised light, photodichroism, and dependence on incident light intensity of the double refringence. Using the angular distribution function (3) these effects can be calculated with standard methods of statistical physics[3].

The magnitude of the photo-orientation of anisotropically absorbing dye molecules can be evaluated at a weak field strength, 3 V cm^{-1}, which corresponds to the incident light intensities given in ref. 2. The damping coefficient, r, can be evaluated from the lifetime of the electronic excitation: $r = 1/\tau$. For dye molecules in a liquid, $\tau \sim 10^{-8} \div 10^{-9}$ s (ref. 2). At room temperatures, when $\tau \sim 10^{-9}$ s the nonlinearity coefficient b is $\sim 10^{-1}$. The corresponding value for the optical Kerr effect in such a weak field is $b_K = \alpha E^2/2kT \sim 10^{-13}$ (ref. 3). At resonance, the polarisability is $\alpha = e^2/4mfr$. Also, $b_K/b = r/f \sim 10^{-6}$. This explains the fact that photo-orientation induced by absorption was observed before the discovery of the laser and before the observation of the optical Kerr effect. The value of the nonlinearity coefficient being known, the photodichroism can be calculated using standard methods[2,3]

$$d = (D_{\parallel} - D_{\perp})/D = -0.5[\langle\cos^2\theta\rangle - 0.5\langle sin^2\theta\rangle] \approx b/15 \qquad (4)$$

where D, $D_{\parallel}$, $D_{\perp}$ are, respectively, the optical densities of natural light, and light polarised parallel and perpendicular to the polarisation plane of the orienting light beam. If $b \sim 10^{-1}$, then $d \sim 10^{-2}$, which is of the same order as the experimental value of photodichroism observed by Neporent and Stolbova[2].

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The electronic structure of VO^{2+}, CrO^{3+}, and MoO^{3+} complexes

THE electronic structures of the d^1 chromophores VO^{2+}, CrO^{3+}, and MoO^{3+} have been the subject of much debate[1–4], particularly after the early molecular orbital (m.o.) calculations of Gray *et al.*[5,6]. An important feature of the electronic spectral assignments suggested[5,6] was that the first two absorptions correspond to the d–d transitions $d_{xy} \rightarrow d_{xz,yz}$ and $d_{xy} \rightarrow d_{x^2-y^2}$, respectively, in the d-orbital sequence d_{xy} (b_2) < d_{xz}, d_{yz} (e) < $d_{x^2-y^2}$ (b_1) < d_{z^2}(a_1). These assignments have found general acceptance and have formed the basis for the interpretation[7] of the electron spin resonance (ESR) spectra and bonding parameters of these complexes. We report here experimental results contradicting the assignment of the second absorption.

We have obtained, for the first time, polarised single crystal electronic spectral data for CrO^{3+} and MoO^{3+} complexes of known structure, including $[(C_6H_5)_4As][CrOCl_4]$, $[MoOCl_3(OP(N(CH_3)_2)_3)_2]$, and $[(C_6H_5)_4As][MoOCl_4(H_2O)]$, at room, liquid nitrogen and liquid helium temperatures. These data, together with certain features apparent[3,4,8] for VO^{2+} complexes, show that several similarities exist between the electronic spectra of VO^{2+}, CrO^{3+}, and MoO^{3+} species (with the possible exception of fluoro- complexes).

(1) The energies of the first two absorptions are characteristic of the MO moiety and are essentially independent of the nature of the other ligands present. VO^{2+} complexes exhibit these absorptions at ~ 13 and 17 kK and MoO^{3+} complexes at ~ 13 and 22 kK. There are few spectral data available at present for CrO^{3+} complexes, but it is tentatively suggested that common absorptions occur at ~ 13 and 18 kK. These observations suggest that the second lowest energy absorption cannot be due to the $d_{xy} \rightarrow d_{x^2-y^2}$ transition as this should be very sensitive to the nature of the ligands *cis* to the M–O group.

(2) These two lowest energy absorptions are polarised. Both are allowed when the electric vector of the incident light is perpendicular to the M–O axis, and forbidden when it is parallel to this direction. Since C_{4v} symmetry is either exact or at least a close approximation for the actual structure of these chromophores, it follows that these two lowest energy electronic transitions must be $E \longleftrightarrow A(B)$ in character. $d_{xy} \rightarrow d_{x^2-y^2}$ is a $^2B_2 \rightarrow {}^2B_1$ transition and is forbidden in both polarisations of C_{4v} symmetry.

(3) The sense of the polarisation and the absolute intensity of the first two absorptions remains essentially unchanged on cooling from room to liquid nitrogen and/or liquid helium temperatures. We therefore conclude that both transitions are electronically allowed. Although $d_{xy} \rightarrow d_{x^2-y^2}$ is symmetry forbidden, it could possibly give rise to an absorption of the observed polarisation by coupling with an E vibrational mode. If this were the case, however, a marked reduction in the intensity and the extent of the polarisation should be observed on cooling.

(4) Fine structure becomes apparent for these first two absorptions on cooling. In each case, the most common spacing of these details are ~ 900 cm^{-1} for the first absorption and ~ 300 cm^{-1} for the second.

To aid the interpretation of the electronic structure of these complexes we have performed *ab initio* m.o. calculations for the ground and excited states of certain CrO^{3+} and VO^{2+} complexes. These results indicate that the lowest energy absorption corresponds to the $^2B_2 \rightarrow {}^2E$ transition, $d_{xy} \rightarrow d_{xz,yz}$, or more correctly $M(d_{xy}) \rightarrow M\text{–}O(\pi^*)$. This interpretation is in agreement with the conclusions of Gray *et al.*[5,6] and all the above experimental data. The fine structure associated with the transition is assigned to vibronic coupling with the M–O stretching mode of the first electronic excited state. In every instance the M–O stretching frequency seems to be less in the excited than in the ground state, as expected in view of the nature of the transition.

The experimental and theoretical evidence available at present for VO^{2+}, CrO^{3+}, and MoO^{3+} complexes gives definite guidance concerning the nature of the second lowest energy absorption, but does not allow for an unequivocal assignment. Self-consistent field calculations[2] for $[CrOF_5]^{2-}$ suggest that this absorption corresponds to a $^2B_2 \rightarrow {}^2E$ electronic rearrangement which is predominantly $M\text{–}O(\pi) \rightarrow M(d_{xy})$ in character, whereas configuration interaction calculations for $[CrOCl_4]^-$ suggest that the transition primarily involves the $^2B_2 \rightarrow {}^2E$ promotion, $M\text{–}O\,(\pi) \rightarrow M\text{–}O(\sigma^*)$, where the latter is the metal–ligand antibonding orbital involving d_{z^2}. Both of these assignments are consistent with points (1)–(4). The origin of the fine structure being attributed[9] to vibronic coupling with the ML_{cis}

B_1 stretching mode, this coupling being necessary[9] to remove the excited state orbital degeneracy. Both suggested electronic transitions have excited state spatial degeneracy within orbitals localised essentially on the oxygen atom, where—in contrast to the first electronic transition where the excited state orbital degeneracy is concentrated on the metal centre—spin–orbit coupling effects are unlikely to remove adequately the spatial degeneracy. Since the calculated ^2E excited state wave functions for the second lowest energy transition are an admixture of several electronic promotions, including the two cited above, it may not prove possible to give a simple description for the origin of this absorption.

The evidence we now have about the electronic structure of VO^{2+}, CrO^{3+}, and MoO^{3+} complexes clearly indicates a considerable similarity in their electronic structure. As generally assumed, the lowest energy absorption is essentially metal $d_{xy} \rightarrow d_{xz,yz}$ in character but the next absorption cannot be due to the $d_{xy} \rightarrow d_{x^2-y^2}$ transition to which it is usually attributed. This latter absorption seems to be due to an oxygen–metal charge-transfer rearrangement within the M–O group. This alternative interpretation destroys the basis used[7] for the interpretation of the ESR spectra of these complxes and thus negates conclusions concerning the nature of bonding parameters so derived.

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Singular behaviour of small crystallites near the melting point

DURING an extension of some recent *in situ* electron microscope investigations into the melting of small particles[1,2], some unexpected effects at temperatures near the melting point were observed for micron-sized crystallites of lead and bismuth. In particular, an unusually enhanced form of diffusion (termed 'snapping') occurred in which many crystallites changed their shapes abruptly into more compact solid forms. Experiments on somewhat smaller crystallites of tin have shown a similar behaviour. Changes of shape during the continuous deposition of thin films in an electron microscope have been reported by other workers[3,4]; these, however, were of a more gradual character, and were consistent with normal diffusion processes.

A second phenomenon observed was the apparent bodily displacement (or 'crawling') of lead crystallites by distances comparable with their length. Small movements of the centre of mass of crystallites due to a change of shape have been noted by other workers[5] and have also been observed in the present work, but these do not involve the

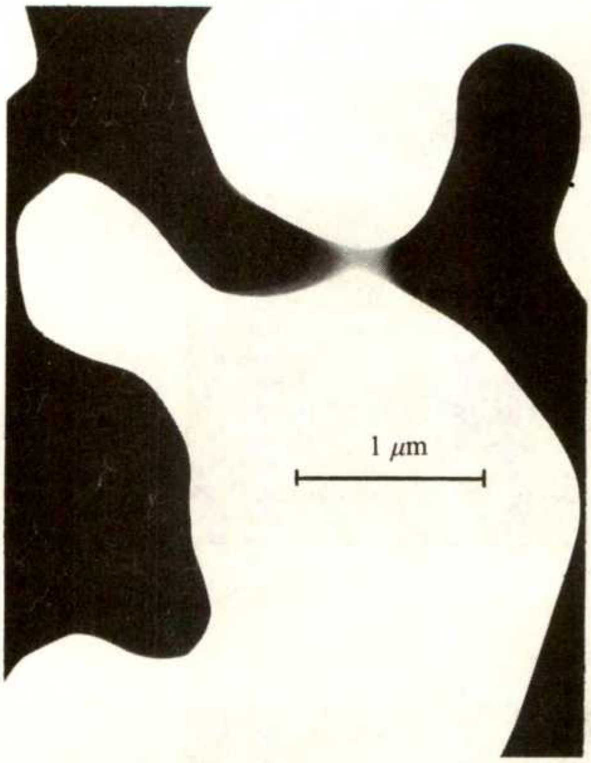

Fig. 1 The rupture, by atomic diffusion, of a neck formed in a thin film of lead (1-s exposure time). Although this diffusion is faster than usual, due to the high curvatures involved, the change is sufficiently slow to cause the edges to be blurred.

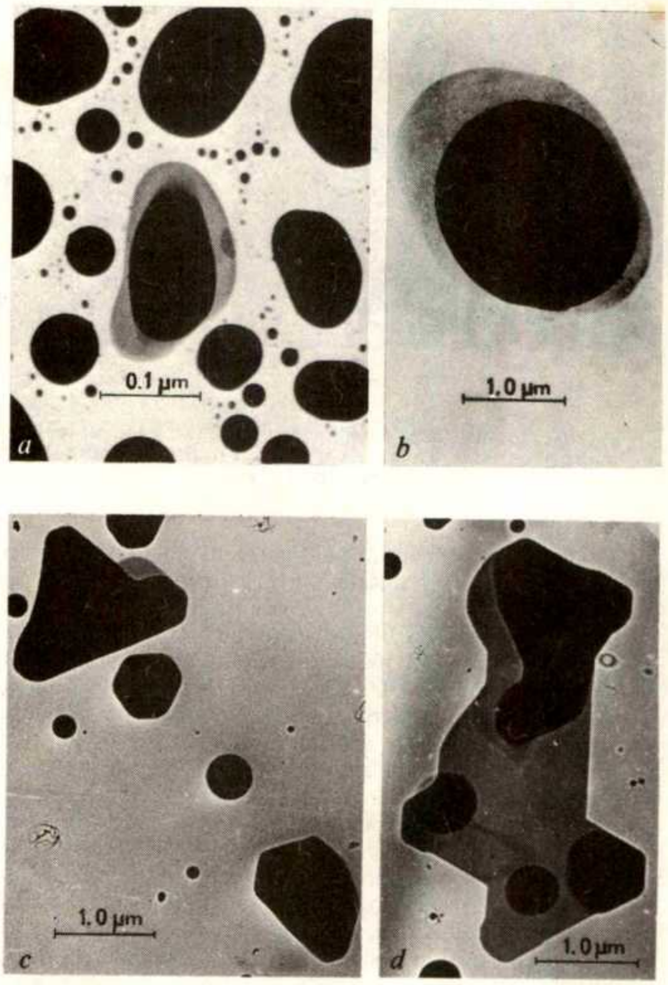

Fig. 2 Snapping in small crystallites (1-s exposure time). *a*, A tin crystallite; *b*, a lead crystallite, showing snapping and subsequent melting; *c*, bismuth particles; *d*, a bismuth crystallite snapping in to two solid and two liquid particles.

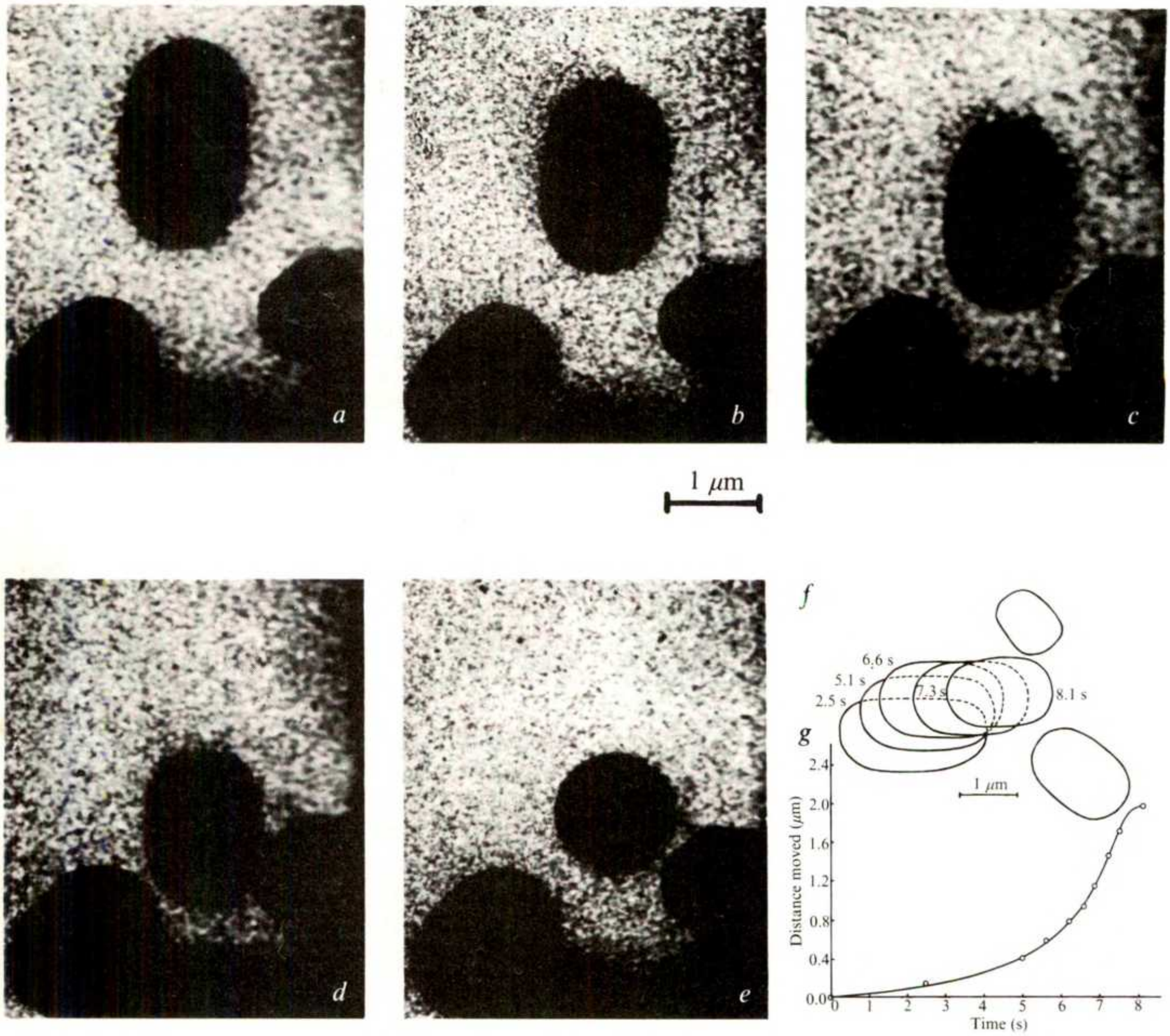

Fig. 3 *a–e*, Frames taken from a cine film showing the movement (and in the final frame the melting) of a lead crystallite relative to some stationary particles. The time with respect to the start of the moment in each case is: *a*, 5.1 s; *b*, 6.6 s; *c*, 7.3 s; *d*, 8.1 s; *e*, 15.6 s. *f*, Scale diagram showing the movement of the crystallite in Fig. 3*a–e*. *g*, Distance of movement plotted against time for the crystallite in Fig. 3*a–e*.

simultaneous movement of all parts of the crystal in a given direction. It is this fact which defines 'crawling' in the present work.

The experiments on bismuth and tin were performed in a JEM 6A electron microscope at a residual gas pressure of about 7×10^{-5} Pa (5×10^{-7} torr), and those on lead in a JEM 100U electron microscope at a residual gas pressure of about 10^{-5} Pa. The specimen films were evaporated *in situ*, usually on to amorphous carbon substrates of graphite, silicon monoxide and partially oxidised aluminium films. After deposition, the specimens were heated at rates ranging from 1 K min^{-1} to 10 K min^{-1}. In general the phenomena described were more pronounced at the faster heating rates. Records were made using either the normal internal plate camera, or a cine camera focused on the screen from a position outside the microscope.

Initially, the lead and bismuth deposits consisted of almost continuous films, containing narrow channels, while the tin deposits comprised individual irregular platelets. On heating, the first noticeable effect was the movement of grain boundaries, resulting in an increase in the size of the grains. In the case of tin, this caused many of the particles to become single crystals, although they retained their irregular shapes. For the bismuth and the lead films, the channels increased in width because of atomic diffusion until the films formed first a network and then (usually within 40 K of the bulk melting point) individual, irregular platelets. This diffusion was slow except in regions of high (convex) curvature, particularly at points where, by the thinning of the material connecting two large particles, 'necks' formed and subsequently ruptured (Fig. 1). The resultant 'needle points' rounded off very rapidly at first, and then more slowly as shapes of lower curvature were formed. As expected, the rate of diffusion was observed to increase with temperature. Little diffusion of this type occurred in the specimens of tin which were already in the form of individual crystallites.

When the films were heated to within a few degrees of the melting point, very abrupt changes of crystal shape were observed to occur for all three materials; this effect was independent of the base used. This was first noticed as the appearance of double images on micrographs taken during the experiments (Fig. 2). During the period of the exposure of the photographic plates (~ 1 s), some crystallites suddenly snapped from a thin form (the larger outlines in Fig. 2) to a more compact form. In other experiments, cine camera records showed no intermediate stage in this process, thus demonstrating the very rapid nature of the change—for example, in the case of bismuth, the change was completed in less than 0.04 s. This behaviour was not necessarily associated with particle forms differing markedly from equilibrium (as, for example, in the necking shown in Fig. 1).

The effect differed slightly for each of the three materials used. For tin (Fig. 2*a*) the change of shape was often symmetric; material from all the side faces building on to the top (and perhaps the bottom) surface. Occasionally, tin particles exhibited a double snapping in which two such abrupt changes occurred one after another, separated in time by ~ 1 s. For lead, the movement was also generally symmetric, but was often followed within a few seconds by melting (Fig. 2*b*). For bismuth the change was, as a rule, asymmetric with the product located towards one side of the initial crystallite. Usually one or more of the original side faces were preserved (Fig. 2*c*) and frequently the snapping resulted in the formation of two or more separated parts, some of which were often liquid droplets (Fig. 2*d*).

When heated to a temperature close to the bulk melting point, several of the lead crystallites were observed to crawl steadily on amorphous carbon substrates; during these movements there were gradual reductions in the projected areas of the particles, which became more compact. Figure 3*a–c* shows a sequence of photographs illustrating the crawling process, and Fig. 3*f* is an outline diagram showing part of the movement, in detail, of the crystallite relative to the surrounding stationary particles. The distance travelled by the centre of gravity of the particle shown in Fig. 3*a–e* has been plotted as a function of time in Fig. 3*g*. The movement of each crystallite generally lasted several seconds, and resulted in a total displacement roughly equal to the length of the particle. The crawling was observed at both high and low electron beam intensities, but was not seen in experiments performed on substrates of silicon monoxide, graphite and partially oxidised aluminium. Crawling was also absent on amorphous carbon in preliminary experiments carried out in a vacuum of about 7×10^{-5} Pa. The liquid droplets obtained after heating to above the bulk melting point showed no sign of such movements.

These experiments are being continued and will be reported in detail in due course.

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Aerodynamic properties of an insect wing section and a smooth aerofoil compared

IN this communication I report measurements of lift and drag forces acting on corrugated aerofoils. The chordal profile of the wings in many orders of insects is corrugated[1] and I have used scale models of a wing section of this type found in the hover fly *Syrphus balteatus* (Diptera, Syrphidae). The experimental profile is shown in Fig. 1*d*. The life-size chord length is 2.9 mm, and occurs 3.7 mm distal to the wing-root hinge, in a total unilateral wing length of 11.5 mm. (There is no special reason either for selecting this particular section or for choosing *S. balteatus*.) The model profiles were 22 times life size (chord length = 6.4 cm) and were made of stainless steel 100 μm thick. They were built to uniform chordal profile, with spans of 7.17 cm or 3.39 cm, giving aspect ratios of 1.12 and 0.53, respectively—both very low. An attempt was made to model the thick leading edge vein of the real wing by constructing the model leading edge from a stainless steel rod (diameter 1.7 mm).

Spanwise corrugation confers a large increase in spanwise stiffness (and allows a decrease in maximum membrane stress when the wing is subject to bending) by comparison with a flat plate of equal plane dimensions and mass. Because of the possibility that corrugations may be seriously detrimental to the aerodynamic qualities of a wing, the aerodynamic properties of the corrugated model were compared with those of another model of the same aspect ratio, of which the chordwise profile was that formed by drawing a smoothed 'envelope' through the corner points of the corrugated section. This is also shown in Fig. 1*d*, and was made of Perspex by grinding and polishing from a larger block.

The lift and drag properties of these models were tested in a small closed-circuit fluid flow channel filled with 45% aqueous

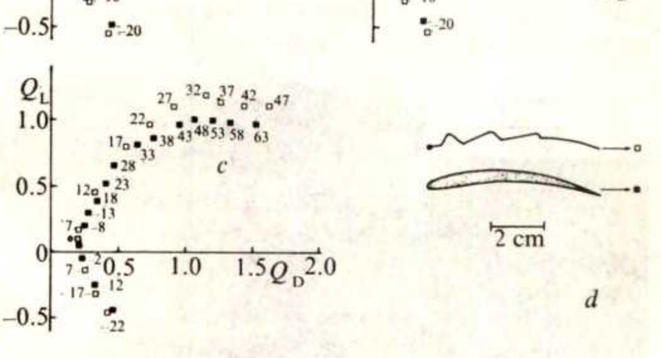

Fig. 1 *a–c*, Polar plots of quasi-lift coefficient Q_L against quasi-drag coefficient Q_D for the corrugated aerofoil (□) and its envelope (■). These profiles are shown in *d*. Small figures by each point refer to the angle of incidence at which that measurement was made. The use of the coefficients Q_L and Q_D and the experimental conditions in which they were measured are explained in the text. *a*, Reynolds number 900, aspect ratio 1.12; *b*, Reynolds number 450, aspect ratio 1.12; *c*, Reynolds number 800, aspect ratio 0.53; *d*, sections of corrugated aerofoil (stainless steel, 100 μm thick, with 2-mm leading edge wire, □) and its solid envelope (Perspex, ■). The corrugated chord section was modelled from a wing profile of the hoverfly *Syrphus balteatus* 3.7 mm distal to the wing root. (See ref. 1.)

sucrose solution. The external plan dimensions of the rectangular circuit were 120 cm × 70 cm, and the channel itself was 12 cm wide and was filled to a depth of 14 cm with the solution. The fluid was propelled round the channel by a 16-blade paddlewheel (diameter 60 cm) running in the long side of the channel opposite that containing the working section. This wheel was powered by a variable speed 500-W electric engine through a reduction gear and pulley system, so that free stream velocities in the range 4 to 25 cm s^{-1} were available. Curved parallel plates were fitted in the channel at its circuit corners, and there were honeycombs just upstream of the working section, which was itself about 8 cm × 11 cm in size. Over this section, the worst photographically measured velocity variations (using very small bubble markers) were -0.4 and $+0.2$ cm s^{-1} at a mean velocity of 8.4 cm s^{-1}, and -0.2 and $+0.3$ cm s^{-1} at 19.8 cm s^{-1}. The temperature of the fluid was maintained at 20 °C, so that the kinematic viscosity of the fluid, υ, was 7.79×10^{-6} $m^2 s^{-1}$ (7.79 cstoke).

The wing sections were mounted on a force balance in which the transducers were two orthogonally bonded pairs of silicon strain gauges (Pye 2A3A120P). When one set of gauges was loaded in its direction of maximum deformation, the other pair recorded a small error signal. The extent of this non-independence was about $+1.8\%$ ("lift" as affecting "drag" sensors) and $+0.95\%$ ("drag" as affecting "lift" sensors), and the results were corrected for this. The wing models were immersed in the flowing fluid such that lift would act perpendicular to the stream in a direction parallel to the channel floor, and drag would act parallel with the stream and the channel floor.

Force measurements were carried out at Reynolds numbers of 900, 800 and 450. These were considered to be values approximately appropriate to the wing of this fly, estimated from relationships given by Weis Fogh[2], and the wing-beat frequency and stroke angle data of Sotavalta[3]. In free flow around an aerofoil, undisturbed by the presence of walls and fluid surfaces, the lift and drag coefficients C_L and C_D may be determined from the expressions:

$$C_L = 2L/\rho u^2 A$$

$$C_D = 2D/\rho u^2 A$$

where ρ is the fluid density, *u* is its free stream velocity, *A* is the projected surface area of one side of the aerofoil perpendicular to chord and span, and *L* and *D* are the forces of lift and drag acting on the wing. In as small a channel as that used here, it is inevitable that there will be interference from the walls and fluid surface, and so the calculated force coefficients should not be interpreted as absolute values. I shall therefore symbolise these quasi-coefficients as Q_L and Q_D, and would stress that they apply to the flow system as described above, and are chiefly of interest from a comparative standpoint.

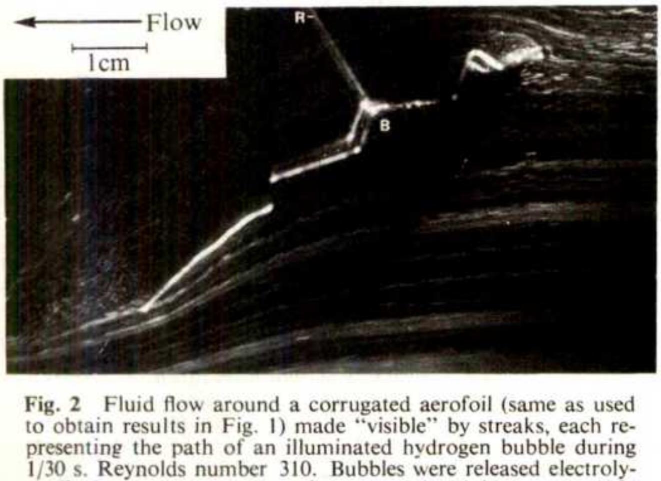

Fig. 2 Fluid flow around a corrugated aerofoil (same as used to obtain results in Fig. 1) made "visible" by streaks, each representing the path of an illuminated hydrogen bubble during 1/30 s. Reynolds number 310. Bubbles were released electrolytically from wire upstream of the aerofoil. Fluid, sucrose 45% (w/v) +0.04% NaCl; kinematic viscosity, 7.79×10^{-6} $m^2 s^{-1}$; aspect ratio, 0.53. R, Supporting wire (diameter 2 mm). B, Region where nearly stagnant fluid is trapped in fold. Incidence angle, 32°.

Conventional polar plots of Q_L against Q_D are shown in Fig. 1. The characteristics of corrugated and smooth envelope aerofoils are similar, especially in the comparisons with the models of higher aspect ratio. No abrupt stall is apparent from the plots at any of the Reynolds numbers, in fair agreement with Vogel's findings[4] for *Drosophila* wings, although the values of Q_L are in general higher than his measurements of C_L. Breakaway was, however, observed at lower angles of attack in regions of the aerofoil away from the tips than near the tips themselves. Figure 2 shows fluid flow around the corrugated section, which has been made visible by means of hydrogen bubbles electrolytically released into the fluid, using a method similar to that of Schraub *et al.*[5]. When the flow near the tip was examined in this way, it appeared that high velocity fluid passing round the wing-tip vortex was preventing the stagnation of fluid in the boundary layer on the upper surface of the wing, and thus eliminating the tendency to stall, up to high (35°) angles of attack; this is seen in Fig. 2 for an angle of incidence of 32°. Breakaway was, however, simultaneously well established at lower angles of attack (about 25°) in regions near the centre of the span of the corrugated or envelope wing with the larger aspect ratio. Instability in flight due to stall will be felt most severely when it occurs at the wing tip. Perhaps the chance of a wing-tip stall is reduced by this form of injection of high velocity fluid, derived from the wing tip vortex. Flow over a corrugated wing seems to involve some fluid becoming trapped in the folds (B in Fig. 2), where it is either stagnant, or rotates slowly about an axis parallel to the span of the wing. The main flow seems to behave as if these folds were solidly infilled and as if the aerofoil were more like the 'envelope' profile.

It thus seems that this wing profile has, at Reynolds numbers of a few hundred, all the advantages of low mass, high stiffness and low membrane stresses in bending which are associated with corrugation, without any obvious aerodynamic shortcoming by comparison with the smooth profile. Perhaps this conclusion may apply also to other corrugated wings which work at Reynolds numbers sufficiently low for the corrugation to present no "roughness" problem.

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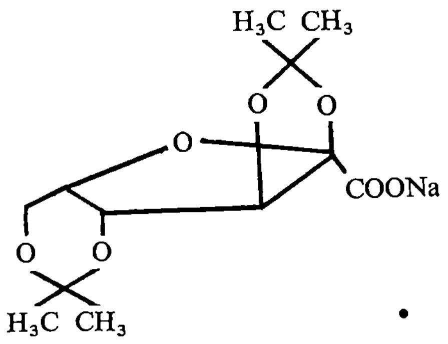
Group of new chemicals with plant growth regulatory activity

THE synthesis of L-ascorbic acid developed by Reichstein and Grüssner[1] is one of very few examples of large scale production of a biologically active monosaccharide. Although the importance of monosaccharides to plants has long been recognised, and several plant growth regulators have been discovered, few of these are carbohydrates and none is a monosaccharide. Active compounds which may be regarded as derivatives of monosaccharides, such as aminoglycosides[2], are chemically too complex to be synthesised on a large scale. We were therefore surprised to find that sodium 2,3:4,6-di-O-isopropylidene-α-L-xylo-2-hexulofuranosonate (I, accepted common name, dikegulac-sodium), an intermediate in the commercial synthesis of L-ascorbic acid, acts as a plant growth regulator.



(I)

To demonstrate the activity of this series of monosaccharide derivatives, Table 1 shows the growth-retarding activity of 11 compounds on *Poa pratensis* L. cv. Norma in the greenhouse. Water-soluble compounds were dissolved in water, and the others were formulated as wettable powders. A spray volume of 1,000 l ha^{-1} and concentrations of 6,000 and 3,000 p.p.m. were used.

The salts were generally more active than the esters although the pentyl and methyl esters were also highly active. As the sodium salt was the most active of the 11 compounds studied, its activity was tested in the greenhouse on a further 10 species (Table 2). Heights of *Lolium perenne* L. cv. Verna, *Digitaria sanquinalis* Scop., *Hordeum vulgare* L. cv. Union, *Malus sylvestris* Mill., *Vitis vinifera* L. Riesling. × Sylvaner and *Liqustrum vulgare* L. were recorded 4 weeks after treatment. The effect of dikegulac-sodium on petiole abscission was tested on *Phaseolus vulgaris* L. cv. Saxa. The plants were cut 1 cm from the main stem and lanolin paste containing the compound was applied to the cut ends. Abscised petioles were counted 6 d later. The effect on fruit ripening was tested on *Lycopersicum esculentum* Mill. cv. Rheinlandsruhm. The plants were sprayed when the first fruits turned red and ripe ones were counted 3 weeks later. Flowers on the first truss of *L. esculentum* Mill. cv. Tiny Tim plants were treated with the test solution using Luckwill's method[3]. Four weeks after treatment the fruits were cut and checked for seed production.

Table 1 Growth retardation activity of dikegulac-sodium and analogues on Kentucky blue grass (*Poa pratensis* L.)

Length 4 weeks after treatment (mm)	p.p.m. 6,000	p.p.m. 3,000
Untreated	197	
-sodium salt	40	40
-ammonium salt	42	52
-calcium salt	48	53
-morpholinium salt	45	45
-methyl ester	48	52
4-*tert*-butylphenyl ester	98	155
-pentyl ester	48	48
-nonyl ester	53	53
2-propynyl ester	53	67
2-hydroxyethyl ester	43	58
4-cyanophenyl ester	65	123

$P = 0.05$; s.e. ±7.0.

Table 2 shows the range of activity of dikegulac-sodium. Growth retardation was seen in a wide range of plants, for example, cereals, cultivated and weed grasses and woody plants. The retardation of *Digitaria sanquinalis* Scop. was sufficient for dikegulac-sodium to be considered herbicidal. The compound induced abscission and stimulated ripening, both of which have been reported to be associated with the plant hormone ethylene[4,5]. The test compound also destroyed apical dominance in *Rhododendron simsii* Planch., inducing the production of axillary shoots and increasing the number of flowers on *Gerbera jamesonii* H. Bolus ex Hook. Dikegulac-sodium also induced parthenocarpic fruit set on *L. esculentum* Mill. cv. Tiny Tim.

Of all the monosaccharide derivatives tested so far in our laboratory, only the diketal analogues of 2-ketogluconic and 2-ketogulonic acids have shown interesting plant growth regulatory activity. Large scale tests have indicated that the most promising effects are the growth retardation and inhibition of apical dominance in woody plants, for example, *Liqustrum vulgare* L., *Thuja occidentalis* L. and *Rhododendron simsii* Planch. It is as yet too early to speculate on the impact which this group of compounds will have on plant production.

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Table 2 Growth regulatory activity of dikegulac-sodium on ten species

Test plants		Length (cm)	No. per plant	s.e.
Hordeum vulgare L.	Untreated	46.1		1.55
	6,000 p.p.m.	37.0		
Lolium perenne L.	Untreated	32.7		1.10
	6,000 p.p.m.	7.0		
Digitaria sanquinalis Scop.	Untreated	24.8		0.98
	6,000 p.p.m.	2.7		
Malus sylvestris Mill.	Untreated	25.2		1.02
	6,000 p.p.m.	15.3		
Vitis vinifera L.	Untreated	72.7		2.46
	1,000 p.p.m.	30.8		
Liqustrum vulgare L.	Untreated	27.3		0.29
	4,000 p.p.m.	12.5		
Phaseolus vulgaris L.	Untreated		0.5*	
	5,000 p.p.m.		1.2*	
Lycopersicum esculentum Mill.	Untreated		9.7†	2.17
	2,000 p.p.m.		16.3†	
Gerbera jamesonii H. Bolus ex Hook.	Untreated		4.6‡	0.37
	200 p.p.m.		6.6‡	
Rhododendron simsii Planch	Untreated		8.5§	0.56
	5,000 p p.m.		13.2§	

* Abscised petioles.
† Ripe fruits.
‡ Flowers.
§ Side shoots.

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Induction of hormone sensitivity by dehydration is one positive role for drying in cereal seed

THE hydrolysis of food reserves in seeds (usually fats, starch and protein) is closely linked to embryo growth since it does not begin until germination has started. The control of food mobilisation by the embryo is best understood in cereals such as barley and wheat. In these cases the hormone gibberellin, moving from the embryo, activates the aleurone layer to produce an array of hydrolytic enzymes, including α-amylase, protease and β-1,3-glucanase and to secrete them into the endosperm where stored materials are located[1]. The stored food is of course laid down in the grain during its development on the mother plant. But at the time when this accumulation is taking place the grains contain relatively high levels of gibberellin[2]; nevertheless, the food reserves seem free from hydrolytic attack. One possible reason for this is that in undried grains on the mother plant the hormonal control system for enzyme synthesis and secretion does not function. No research has been done directly on this problem; what relevant research there is shows some discrepancies[3,4]. Our results, described here, demonstrate that the hormonal control system operates fully only after the mature grains have been dried. Dehydration is necessary for the development of complete sensitivity to gibberellin.

Barley (*Hordeum distichum* cv. Julia) was grown in the greenhouse from December 1974 to March 1975 with supplementary lighting to give a 16-h daily light period, or in the field at the Lord Rank Research Centre, High Wycombe. Grains were collected approximately 30 d after anthesis. The grains were dehulled (fresh weight 60± 3 mg per seed) and half the sample was left to dry at room temperature for 2 d when a weight of approximately 31 mg per seed was reached (a water content of 9 6%). Embryos were removed from the dried and undried samples and the embryoless grains were sterilised in sodium hypochlorite (1.4% available chlorine) for 20 min. They were then rinsed several times and incubated according to the method of Jones and Varner[5] but with the medium containing chloramphenicol (10 μg ml^{-1}) and nystatin (100 U ml^{-1}). Gibberellic acid (10^{-6} mol l^{-1}) was included in the flasks. Incubation was terminated at different times and the medium was assayed for α-amylase[5] and β-1,3-glucanase[6]. These enzymes were chosen because α-amylase is both synthesised and secreted in response to gibberellic acid, whereas the β-1,3-glucanase requires the hormone only for its secretion. Thus, the two hormone-controlled processes, enzyme synthesis and enzyme secretion, could be studied separately. The aleurone layers were removed from the grains without embryos for extraction of any non-secreted enzymes remaining in the cells.

The results for α-amylase (Fig. 1) show the characteristic hormone-induced enzymatic response in the case of the previously dried grain, but the undried grains fail to respond until after extremely long exposure to gibberellic acid and then to only a small degree. Increased response by undried grains can, however, be provoked by very high gibberellin concentrations but even so, enzyme synthesis and secretion at the level exhibited by dried seeds is never attained (Table 1). The degree of sensitivity of barley aleurone cells to gibberellin therefore clearly depends on previous dehydration of the grain. The findings suggest that there would be no appreciable breakdown of starch during its accumulation in the developing grain as the hormonal control system for enzyme production would be inoperative. The biological importance of this is clear. The practical implications are connected with the spoilage of dried cereal grain when this is rewetted before harvesting; here, hormone-controlled enzyme production can occur, leading to starch hydrolysis.

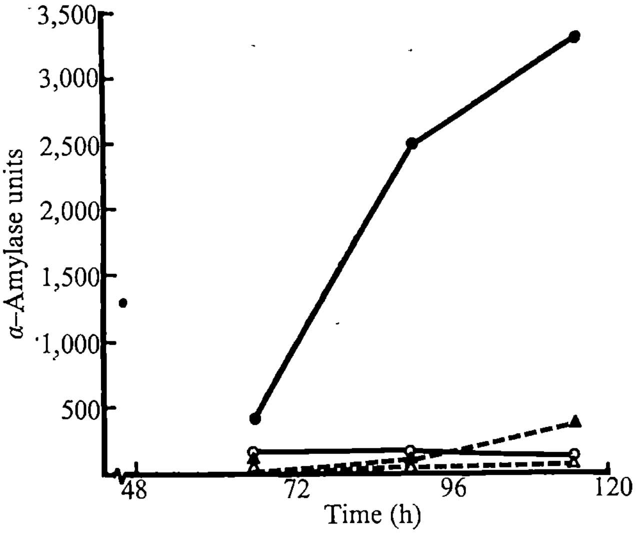

Fig. 1 α-Amylase production by dried and undried half grains. Ten half grains were incubated for appropriate lengths of time in 2 ml of medium. After incubation, medium was decanted, half grains were washed with 2 ml of buffer and washings were added to medium. After centrifugation supernatant was assayed for secreted enzyme. Aleurones were removed from washed half grains and homogenised in 4 ml of buffer. After centrifugation supernatant was assayed for non-secreted enzyme. ———, Dried grains; – – – –, undried grains; ● and ▲, secreted enzyme; ○ and △, non-secreted enzyme.

The pattern for β-1,3-glucanase is different, however, since dried and undried grain show only slight differences in total enzyme (Fig. 2) but drying does have some relatively small effect on the gibberellin-sensitive secretory mechanism. Thus the indication is that secretion can function almost fully even in seeds which have not been dehydrated. The results for

Fig. 2 β-1,3-Glucanase production by dried and undried half grains. Half grains were incubated and enzymes extracted as described for Fig. 1. Symbols as in Fig. 1.

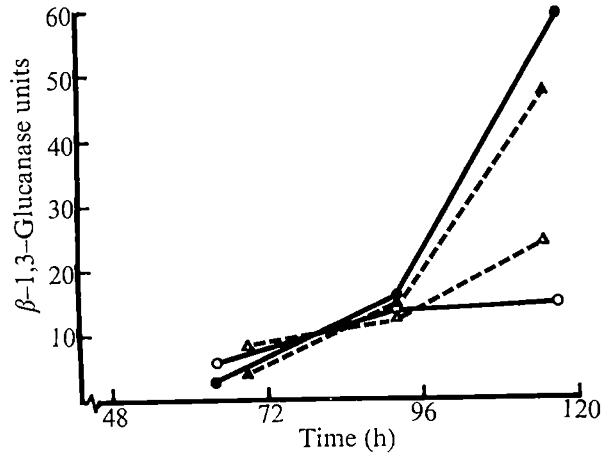

Table 1 Effect of gibberellic acid concentrations on α-amylase secretion in undried grains

Gibberellic acid concentration (mol l^{-1})	α-Amylase units (per 10 embryoless grains)
10^{-6}	420
10^{-5}	900
10^{-4}	1,000

Ten half grains were incubated for 114 h in 2 ml of medium containing various concentrations of gibberellic acid. After incubation, medium was decanted, half grains were washed with 2 ml of buffer and washings were added to medium. After centrifugation, the supernatant was assayed for enzyme activity.

α-amylase and β-1,3-glucanase together indicate that previous drying is required probably only for hormone-induced synthesis (for example, α-amylase) and not for enzyme secretion.

The control of hormonal sensitivity by dehydration is matched by the effect of this treatment on germination itself[7]. In our experience undried, intact grains as collected for the above experiments do not germinate, but when dried for 2 d the rewetted seeds germinate within only 3 d at 20 °C.

In all, our results demonstrate the critical importance and positive role of dehydration in the physiology of a cereal seed. These findings demonstrate (apparently for the first time) how the full operation of a hormonal system in a plant tissue is instigated by previous drying. As far as the biology of seeds is concerned we should see this as a device for retaining the integrity of the food reserves until dispersal from the mother plant has been achieved. Together with this, the continued growth of the embryo (that is, germination) is restrained and can recommence only after drying has been experienced. The physiology of these two aspects of the controlling role of dehydration may be basically the same, connected with the regulation of the synthesis of enzymes, in one case for food reserve hydrolysis, in the other for germination itself. Experiments are in progress to investigate these phenomena further.

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Alteration of epididymal sperm transport and maturation in mice by oestrogen and testosterone

THE maturation of spermatozoa during passage through the mammalian epididymis, involves changes in motility, metabolism, morphology, biochemical properties and the development of the ability to fertilise ova[1]. This maturation process, and the maintenance of fertilising ability, is dependent on androgen[2]. When testosterone levels are lowered by castration or hypophysectomy, sperm from the cauda epididymis have immature acrosomes (guinea pig)[3] and lowered fertilising ability (rat)[4]. These effects are prevented by testosterone treatment. Since reduction of testosterone levels enhances the rate of epididymal sperm transport[4,5], the incomplete maturation could result from spermatozoa spending insufficient time in critical regions of the epididymis, as well as from a decreased ability of the epididymis to promote sperm maturation. We demonstrate here that oestrogen and testosterone affect one aspect of sperm maturation in mice by altering the rate of epididymal sperm transport, and also affect fertility.

Male BC3F$_1$ hybrid mice were injected daily with either 10 μg d^{-1} oestradiol benzoate (OB), 80 μg d^{-1} testosterone propionate (TP), both hormones (OB+TP) or sesame oil alone. Administration of these hormones for up to 50 d produced negligible effects on the process of spermatogenesis in the testis (Table 1, Fig. 1). This observation is not inconsistent with other studies; the inhibition of spermatogenesis in mice by oestrogens is strain dependent[7].

Table 1 Sperm content of testes, epididymides and vasa deferentia of hormone-treated mice (sperm heads per mouse $\times 10^{-6}$)

	Control	OB	OB+TP
Testes	51.6±1.9	51.9±1.7	47.2±2.1
Capita epididymides	20.6±0.8	1.4±0.2*	7.2±0.4*
Corpora epididymides	6.7±0.8	2.0±0.2*	3 8±0.2*
Caudae epididymides	49.2±1 4	10.8±1.3*	27.8±1.0*
Vasa deferentia†	3.6±0.6	4.0±0.6	2.9±0.4

BC3F$_1$ male mice (C57BL ♀×C3H ♂ from Cumberland View Farms, Clinton, Tennessee) were injected daily with hormone given intramuscularly in the leg in 0.05 ml of sesame oil for 39–50 d. Oestradiol benzoate (OB) was obtained as Oestroform (British Drug Houses); testosterone propionate (TP) as Perandren (Ciba). The sperm contents of these organs were determined by counting the number of sperm nuclei ('sperm heads') in sonicated tissue homogenates in a haemocytometer. The spermatid nucleus becomes resistant to sonication at late step 12, and remains sonication resistant throughout further maturation[6]. The sperm heads were prepared by homogenisation of the tissue in distilled water with a Polytron homogeniser, sonication and filtration through 10-μm pore nylon cloth, to remove debris.

* Significantly different from controls at $P<0.01$.

† In other experiments, OB significantly reduced the number of sperm in the vas, as well as in the epididymis, while having little effect on number of sperm in the testis.

In contrast, the sperm content of the epididymis was markedly reduced by OB treatment (Table 1). Treatment with OB+TP produced a smaller reduction. TP alone had little effect. Except for the decrease in sperm content and for a small, but significant, reduction in the height of the epithelial cells, the histological appearance of the epididymis was unchanged by OB treatment.

The reduced numbers of spermatozoa could result from lower testicular sperm production, resorption of spermatozoa in the epididymis, or an enhanced rate of sperm transport through the epididymis. Testicular sperm production was calculated by dividing the number of sonication-resistant sperm heads (Table 1) by the time span (5.9 d)[6] from late step 12 through further maturation[8]. Similar values of 8.7×10^6, 8.8×10^6 and 8.0×10^6 sperm d^{-1} were obtained for control, OB and OB+TP treated mice, respectively.

Experiments with mice, unilaterally vasectomised for 6–10 d, demonstrated that resorption of sperm in the epididymis is negligible. The accumulations of spermatozoa in the ligated-side epididymides of these mice were 11×10^6, 6.3×10^6 and 6.8×10^6 sperm d^{-1} for the 2 epididymides in control, OB and OB+TP treated mice, respectively, closely matching the sperm production rates. In addition, the reduction in the total radioactivity of the sperm in the reproductive tract, which occurred between days 27 and 31 in OB treated mice as the radioactively labelled sperm passed out of the vas, was largely prevented by ligation of the vas.

The kinetics of the transport of spermatozoa from the testis through the epididymis and the vas deferens was studied by measuring the radioactivity in cell suspensions or in sperm nuclei from different segments of the reproductive tracts of mice,

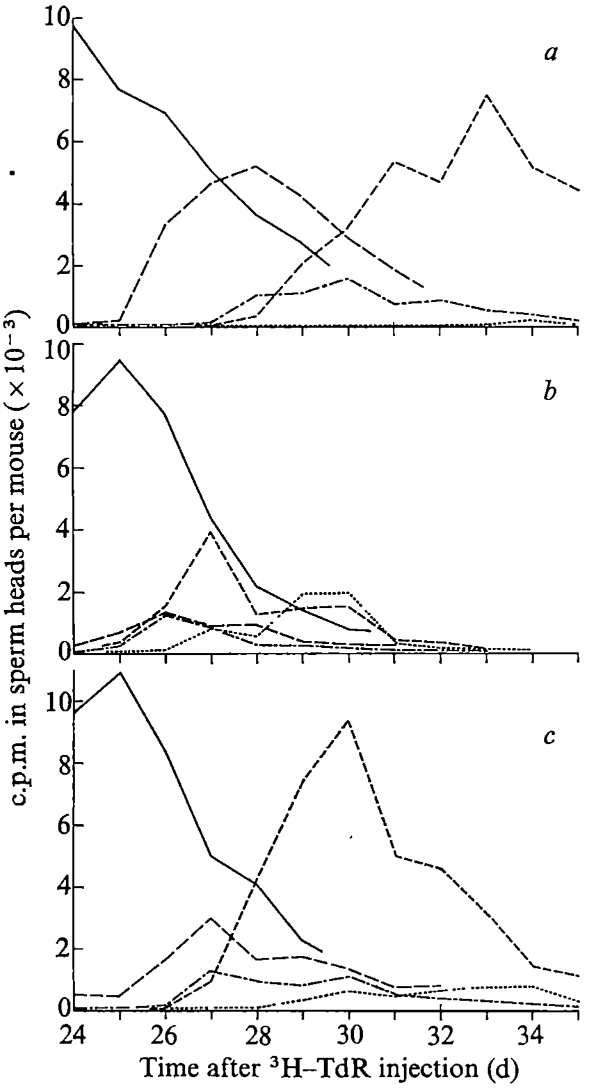

Fig. 1 Passage of radioactively labelled spermatozoa through the male reproductive tract of control (*a*), OB-treated (*b*) and OB+TP-treated (*c*) mice. Mice were given daily injections of the indicated hormone. On day 15 of hormone treatment, they were injected intraperitoneally with 30 μCi of ^{3}H-thymidine (TdR). Sperm heads, prepared from the testis (———), caput (— —), corpus (— - —), cauda epididymis (- - -) and vas deferens (···) of mice killed on the indicated days, were collected by filtering onto Whatman GF/C filters. The radioactivity in the sperm heads was determined by liquid scintillation counting (30% efficiency). Data points (except for day 35) are averages of 3 samples, each prepared from one mouse. The average fractional standard errors in the counts between animals were 0.11, 0.15, 0.22, 0.24, 0.26 for the testis, caput, corpus, cauda epididymis and vas deferens respectively.

killed at daily intervals after injection of ^{3}H-TdR. The radioactivity in the testis was constant for 25 d after the administration of ^{3}H-TdR (data not shown), and then decreased similarly in all experimental groups (Fig. 1). In control and TP-treated mice, radioactive spermatozoa first entered the caput epididymis on day 26, the corpus on day 28, the cauda on day 29, and the vas not until day 34, or later. In OB and OB+TP-treated mice, however, radioactively labelled spermatozoa were present in the vas deferens on days 27 and 29 respectively.

The epididymal transit times calculated from these profiles clearly show a marked reduction with OB treatment and a less marked reduction with OB+TP treatment (Table 2). The reductions in epididymal sperm counts are a result of this increased transport rate, since transit times calculated from the sperm counts are nearly identical to those from radioactivity profiles.

Table 2 Average epididymal transit times in hormone-treated mice (d)

Epididymal segment	Control	OB	OB+TP
Caput	2.3*(2.4)†	0.8 (0.2)	1.5(0.9)
Corpus	1.6 (0.7)	−0.1 (0.2)	0.5 (0.5)
Cauda	⩾5.8 (5.6)	1.2 (1.2)	1.8 (3.6)

* Calculated from radioactivity profiles (Fig 1). The average transit time for a given segment is the difference between the times at which labelled spermatozoa enter that segment and enter the next segment (start of rise of curves), or, equivalently, the difference between the times the spermatozoa leave the previous segment and leave that segment (maxima of curves) Unbiased estimates of these times were obtained by computer fitting the curves with exponential functions (manuscript in preparation). All values are averages of determinations from the times of entry and of leaving. For the control cauda epididymis, lower limits were chosen assuming labelled spermatozoa enter the vas after day 35.

† Calculated from sperm counts (Table 1). In the absence of sperm resorption, the same number of spermatozoa per day must pass through each point in the reproductive tract and that number equals the testicular sperm production rate (S) The number of spermatozoa present in any segment (n), equals the rate of entry of spermatozoa (S) times the average time a spermatozoon spends in that segment (t), which is the transit time. Therefore, $t = n/S$.

Experiments were then performed to determine if one aspect of sperm maturation, disulphide crosslinking of the sperm nuclear protein[9], was affected by the hormonal treatment. Mouse sperm nuclei show progressive maturation during passage through the epididymis (Table 3). Sperm nuclei from each epididymal region of OB-treated mice were, however, less mature than those of control mice. This was not a direct result of OB treatment on sperm maturation, but rather a consequence of the enhanced rate of transport. Spermatozoa, blocked, by vasectomy, in the cauda epididymis and proximal region of the vas deferens of the OB-treated mice, showed increased disulphide crosslinking. This aspect of sperm maturation is time dependent, not necessarily dependent on the epididymal milieu, as expected from previous observations[9].

The crucial aspect of sperm maturation is the development of fertilising ability. We tested mice, given hormonal treatment for 28 d before and during mating, by placing each male with two virgin females for two weeks. The males were then killed, and showed epididymal sperm counts, unaltered by mating. The females who did not bear litters were killed 14 d later, and checked for the presence of embryos. Nine out of nine control mice were fertile, but only two out of nine OB+TP-treated mice were fertile. All nine mice injected with OB were sterile. The fertility of the treated mice is significantly below that of controls ($P<0.005$). Vaginal plugs were seen in females in all experi-

Table 3 Effect of hormonal treatment on sperm head maturation, measured by times (min) of incubation in SDS–DTT required for swelling

Source of sperm heads	Control	OB	OB+TP
Caput epididymis	12±1	2	—
Cauda epididymis	21+1	11±1*	20±0.3
Vas deferens	26±0.5	19±1†	—
Cauda-proximal vas from vasectomised mice			
Control side	26	12±1	—
Vasectomised side	22	30±3*	—

All mice were killed after 45–47 d of hormone treatment. Several mice were unilaterally vasectomised after 40 d of hormone treatment by ligating and cutting the vas deferens.

The extent of formation of disulphide linkages in the sperm nucleus was determined by the rate of swelling in 1% sodium dodecyl sulphate (SDS) and 2×10^{-3} M dithiothreitol (DTT) in 0.05 M sodium borate buffer (pH 9.0)[9]. Sonicated sperm heads were suspended in SDS–DTT and observed microscopically during incubation at room temperature (24±1 °C) from 2 to 30 min The level of swelling was scored on a 0–4 scale, and the time required to reach the level of 2 was determined by interpolation.

* Significantly different from controls at $P<0.01$. In vasectomised mice, the statistical comparison was made between vasectomised and control sides.

† Significantly different from controls at $P<0.05$.

mental groups. Further experiments are needed, however, to determine whether the infertility of OB-treated mice is a result of the immaturity of the sperm, or some other effect of the treatment.

The enhancement of epididymal sperm transport by oestrogen is a novel finding. The slowing of sperm transport by testosterone had been reported previously in the rat using ligation of the efferent ducts and/or vas deferens[4,5]. Here, we confirm this phenomenon in mice without using ligation, which might perturb fluid and blood flow. The mechanisms responsible for sperm transport and its hormonal regulation are not known. Possibilities include peristaltic contraction of the epididymal tubules and action of the ciliated cells of the epididymal epithelium[10]. These mechanisms, however, seem to be inconsistent with our findings, since testosterone enhances epididymal contractions[10] and the size of stereocilia[11]. Our observation that the effect of OB is decreased by additional treatment with TP, indicates that OB may act to interfere with either the endogenous production or action of androgen. Oestrogen can reduce testosterone production by direct action on the Leydig cells, or by inhibiting the secretion of luteinising hormone[12]. Morphological changes in the Leydig cells, which accompany depression of luteinising hormone levels[13], were, however, not observed in OB-treated mice. Alternatively, oestrogen can act directly on the epididymis to reduce the effects of testosterone[14], either by competitive binding to a testosterone receptor[15], or by binding to an oestrogen receptor[16] to produce a change opposite to that of androgen.

The results presented suggest the possibility of the regulation of male fertility with oestrogenic or antiandrogenic hormones. Such hormones could, without affecting testicular function, act on the epididymis to enhance the rate of sperm transport, resulting in immature spermatozoa in the ejaculate.

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Reduced deformability of erythrocyte membranes from patients with Duchenne muscular dystrophy.

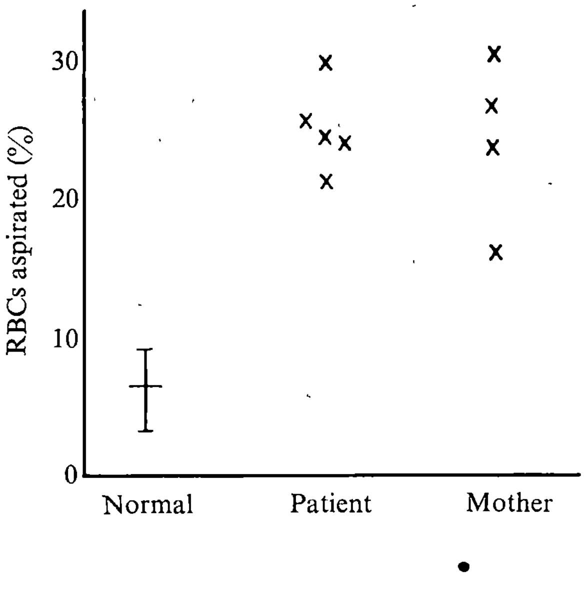
THE fundamental defect in Duchenne muscular dystrophy is obscure. Early investigators attributed the disorder to a primary defect of the skeletal muscle itself,[1] but alternative suggestions have implicated the motor neurone[2,3] or the vascular supply[4]. Recent attention has been directed to the plasma membrane of muscle as well as of other tissues. Several authors have postulated a generalised membrane defect as the fundamental problem in this disorder[5–7].

Matheson and Howland[6], using a scanning electron microscope, examined the erythrocytes of patients with various forms of muscular dystrophy. When compared with control erythrocytes, the patients' cells exhibited striking alterations in surface contour. These changes were most prominent in cells derived from Duchenne patients. The obligate female carriers for Duchenne dystrophy also showed similar deformities of the erythrocyte surface. It is clear that these surface changes are not specific for the muscular dystrophies as such changes have also been noted clinically in association with severe liver disease, uraemia, splenectomy, haemoglobinopathies and β-lipoprotein deficiency. It was previously noted by Weed and Chailley[8] that similar erythrocyte alterations could be induced in 'normal' erythrocytes by incubation in the presence of the divalent cation, calcium, or in solutions whose *p*H exceeded 8.5. The resulting cells, echinocytes, have also been produced following incubation of erythrocytes in the presence of a variety of chemical compounds including salicylates, barbiturates, and 2,4-dinitrophenol.

On the basis of observations of Matheson and Howland, we speculated that the altered surface contour of the Duchenne patients' erythrocytes may be reflected in abnormal membrane elasticity or deformability. To evaluate this property, we have used cell elastimetry modified from the original method of Mitchison and Swann[9]. This method has been used successfully in our laboratory in the study of polymorphonuclear leukocytes[10] and was readapted readily to the study of erythrocytes.

Heparinised blood was obtained by venipuncture and the plasma removed by centrifugation. The cells were then washed three times in a balanced salt solution at *p*H 7.2. Micropipettes were pulled to an internal diameter of 1–2 μm and attached to a sensitive source of negative pressure. With microscopic assistance, a micromanipulator was used to direct an erythrocyte to the pipette orifice. The amount of negative pressure

Fig. 1 Percentage of erythrocytes aspirated at negative pressures greater than 100 mmHg in preparations from controls, Duchenne dystrophy patients and obligate female carriers.



required to aspirate the erythrocytes into the pipette was determined. Measurements were not carried beyond negative pressures of 250 mmHg even though some cells were not aspirated at negative pressures exceeding 500 mmHg. The negative pressure required to aspirate an erythrocyte into the pipette could depend either on the fluidity of the cytoplasm or the plasma membrane. The latter seems more likely since red cell ghosts have been shown to exhibit the same deformability characteristics as intact erythrocytes[11].

Negative pressure measurements were carried out on 100 red cells from each control or patient and the percentage of cells aspirated at negative pressures in excess of 100 mmHg was computed.

We have used this technique on erythrocytes from five patients with Duchenne muscular dystrophy and four obligate female carriers. All diagnoses were substantiated by abnormal serum creatine phosphokinase (CPK) levels, muscle biopsy findings, and genetic analysis.

Of the Duchenne dystrophy erythrocytes, 25% were aspirated at negative pressures greater than 100 mmHg compared with only 6% of control erythrocytes (Fig. 1). It is interesting that erythrocytes from obligate female carriers exhibited a comparable reduction in deformability. Both observations are highly significant (Student's *t*-test).

The relevance of these observations may be considered from two perspectives. First, in terms of deformability, erythrocytes from Duchenne patients are easily distinguished from control cells as are those from the obligate female carriers. Since the measurement of serum CPK levels fails to delineate carriers consistently, the biological assay used in this study may offer an effective adjunct for carrier detection, and thus, for genetic counselling.

Second, and more fundamental, is the relevance of altered erythrocyte deformability in Duchenne dystrophy. To address this question, it is important to examine the mechanism of this deformability. Phagocytic cells, including PMNS have a recognisable microtubule–microfilament system distributed beneath the plasma membrane. Abundant data indicate that this system involves contractile proteins similar to, if not identical with actin and myosin. Recent evidence from our laboratory has indicated an association between membrane deformability and microtubule–microfilament function in PMNs[12]. Although erythrocytes lack a microtubule system as such, proteins with a fibrillar appearance have been demonstrated on the cytoplasmic surface of the red cell membrane. These proteins are myosin- and actin-like and exhibit ATPase activity characterised as ouabain insensitive and divalent cation dependent[13,14]. These properties are analagous to those of myo-fibrillar ATPase and to mitochondrial ATPase. This association of ATPase activity with fibrillar proteins on the inner surface of the red cell membrane provides a reasonable basis for understanding the process of erythrocyte membrane elasticity or deformability.

The observations of this study support the present notion of a generalised membrane defect in Duchenne dystrophy and suggest that further studies of the biochemical events associated with the dynamic process of red cell deformability may well explain the basic defect in this disorder.

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Enhanced *Trypanosoma musculi* infections in mice with concomitant malaria

A PHASE of immunodepression is characteristic of experimental malaria infections and, in mice, immune responses to antigens as diverse as sheep red blood cells[1], tetanus toxoid[2], oncogenic viruses[3] and *Toxoplasma gondii*[4] are diminished. From immunological and epidemiological viewpoints it is important to know what happens when animals are infected with another agent during this period of immunodepression. The agent must be carefully chosen; it must be able and likely to coexist with the malaria parasite *in vivo*, be easy to monitor and elicit a well defined immune response *Trypanosoma musculi*, an avirulent murine trypanosome, fulfils these criteria. There is no cross

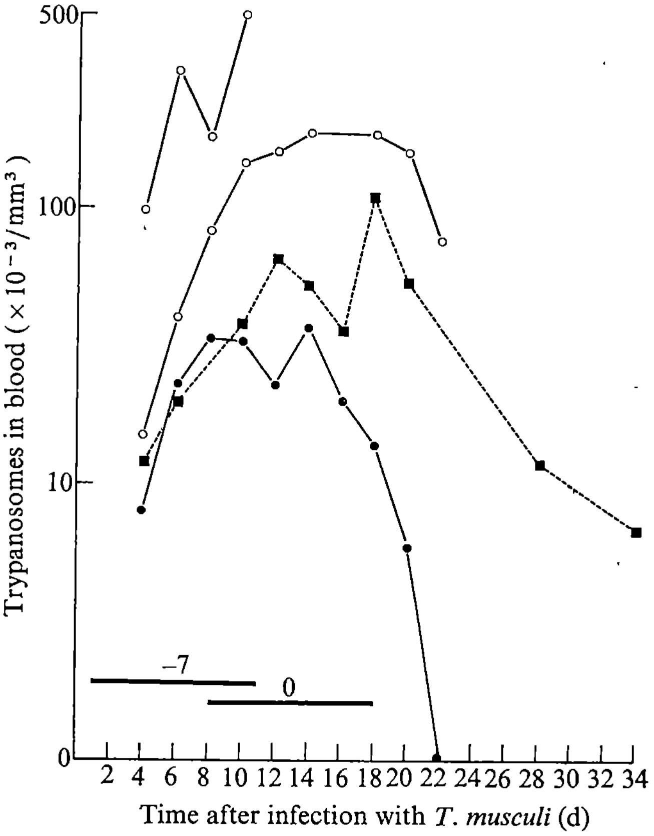

Fig. 1 Patterns of infection with *T. musculi* in female TO Swiss mice Each point represents the mean of all control mice (●), mice infected with *P. yoelii* at the same time (■) and mice infected with *P yoelii* 7 d earlier (○). This last group has been divided into subgroups; in one of these 5 mice died of trypanosomiasis within 10 d whereas the remaining 13 survived for at least 21 d. The graph is incomplete because the death of some mice after day 22 and the fact that some mice had recovered from their infections while others still harboured parasites, would give a false impression of the nature of the infection during its final phase if the mean parasitaemias were plotted. This latter point also applies to the other experimental group. The solid blocks, −7 and 0, indicate the periods of immunodepression related to infection with *P. yoelii* 7 d before *T musculi* and at the same time.

immunity between it and rodent malarias[5], the immune response has been very carefully characterised[6–8] and the progress of the infection can be monitored easily by taking minute quantities of blood. We have found that *T. musculi* infections are considerably enhanced in mice coincidentally infected with an avirulent malaria parasite, *Plasmodium yoelii* (*P. berghei yoelii*)[9].

Batches of mice were infected intravenously with approximately eight *P. yoelii* parasites and challenged by the intravenous injection of about 1,000 *T. musculi* at the same time or 7 d later. The trypanosome infections were monitored and the results are shown in Fig. 1. Infections in 18 control mice resembled those described by Viens *et al.*[6] and the mice overcame the infections within 22 d. In all the experimental animals, the infections were more intense and persisted for longer than in the controls. The greatest enhancement occurred in mice infected with *P. yoelii* 7 d earlier. In this group, 5 out of 18 mice died of trypanosomiasis within 10 d, one mouse harbouring over 5×10^5 trypanosomes per mm^3 of blood just before it died. Of the remaining mice, a further four died on days 22–24 of a combination of malaria and trypanosomiasis, two died of recrudescent malaria 25 and 26 d after infection while still harbouring some trypanosomes and seven became negative between days 21 and 28. In 18 animals infected with *T. musculi* and *P. yoelii* at the same time the infections resembled those in control mice until day 10 but thereafter they continued to rise for a further 7–9 d before gradually declining until all the mice were negative on day 41. There were no fatalities in this group.

Immunodepression during *P. yoelii* infections coincides with the peak of parasitaemia and lasts for about 10 d (ref. 1). In mice infected with *P. yoelii* 7 d before *T. musculi* this period of immunodepression would have occurred during days 1–11 of the trypanosome infection, just when the control animals were beginning to overcome it. In mice infected with *P. yoelii* at the same time as *T. musculi* the period of immunodepression would have extended over days 8–18 and this is consistent with the observation that it is during this period that the infections in the experimental mice diverge from those in the controls.

Immunity to *T. musculi* has been shown to operate in two stages[6–8]. The first response after about 6 d inhibits reproduction and involves T-cell dependent ablastin and T-cell independent trypanocidal antibody. The second response brings about the elimination of the trypanosomes and is T-cell dependent. In T-cell deprived mice, *T. musculi* infections persist for the lifetime of the host[6]. This was not the case in our experiments and our results indicate that *P. yoelii* produces a defect in the first response. There is, as yet, no satisfactory explanation of immunodepression during malaria infections but a hypothesis which suggests that antibody producing cells, particularly those responsible for IgM, are affected is consistent both with our findings and with the well known fact that cells forming plaques to sheep red blood cells are considerably reduced during *P. yoelii* infections[1].

The epidemiological significance of immunodepression during malaria is considerable because the chances of transmission of a superimposed infection increase as the intensity and duration of that infection increase. There are also reciprocal effects between these parasites. In these experiments we have noted that *P. yoelii* infections are enhanced in some, but not all, mice infected with *T musculi* with the result that mice may die unexpectedly from recrudescenses of malaria or from the cumulative effect of the dual infection. We have also observed that the piroplasm *Babesia microti* enhances *T. musculi* infections and that both *P yoelii* and *B. microti* modify *T. brucei* infections.

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T-cell activation in murine malaria

THE immune response of rodents to malaria is thymus dependent. *Plasmodium berghei* infections are more severe in neonatally thymectomised rats than in intact controls[1], whereas *P. yoelii (P. berghei yoelii)* infections which are self-resolving in normal CBA mice prove fatal in T-cell deprived CBA mice (Jayawardena *et al.*, unpublished) or congenitally athymic nude mice[2]. Malarial infections are also known to depress immune responses to certain antigens. In the mouse, they reduce the antibody[3] and splenic plaque forming cell[4] response to sheep red blood cells (SRBCs), render animals more susceptible to lymphomagenic viruses[5], and severe long term infections prolong xenogeneic skin graft survival time[6]. It has been suggested that such impaired immune reactivity reflects defective lymphocyte or macrophage function, but there has been no unequivocal evidence in support of these possibilities.

We have examined the T-cell mitotic response, the production of anti-parasite antibodies and parameters of T-cell function (the response to phytohaemagglutinin (PHA) and oxazolone) in non-fatal *P. yoelii* and fatal *P. berghei* infections in the CBA mouse, and present evidence suggesting that the development of protective immunity and the nonspecific immunodepression can be related to the T-cell response of the host.

We defined the T-cell response to infection in CBA/Lac–CBA/T_6T_6 mouse radiation chimaeras carrying a chromosomally distinguishable population of T cells. To prepare these radiation chimaeras CBA/Lac mice were thymectomised at 7 weeks of age, subjected to 850 rad X irradiation 10 d later, and reconstituted immediately with 5×10^6 femoral CBA/Lac bone marrow cells. Within 20 h of X irradiation, thymus grafts consisting of two thymus lobes from CBA/T_6T_6 neonates were implanted under the kidney capsule[7]. The grafts remained *in situ* for 30 d after which they were removed[8]. Twenty days later, these mice were infected with the 17X strain of *P. yoelii* or the Anka strain of *P. berghei* (all infections were initiated with an intraperitoneal inoculum of 1×10^5 parasitised RBCs) At various times after infection the spleens were removed and processed for cytological analysis[8] and the proportion of dividing cells, known by their chromosome marker to be of thymus graft origin, was determined. (A minimum of 100 dividing cells were scored at each stage of infection)

The patterns of parasitaemia and the T cell mitotic response in the spleens of infected mice are shown in Fig 1. In mice infected with *P. yoelii*, parasites were detected in the peripheral blood within 2 d and peak parasitaemias were recorded on day 10, after which the infection regressed, all mice being aparasitaemic by day 18 Within 4 d of infection (when the percentage parasitaemia was

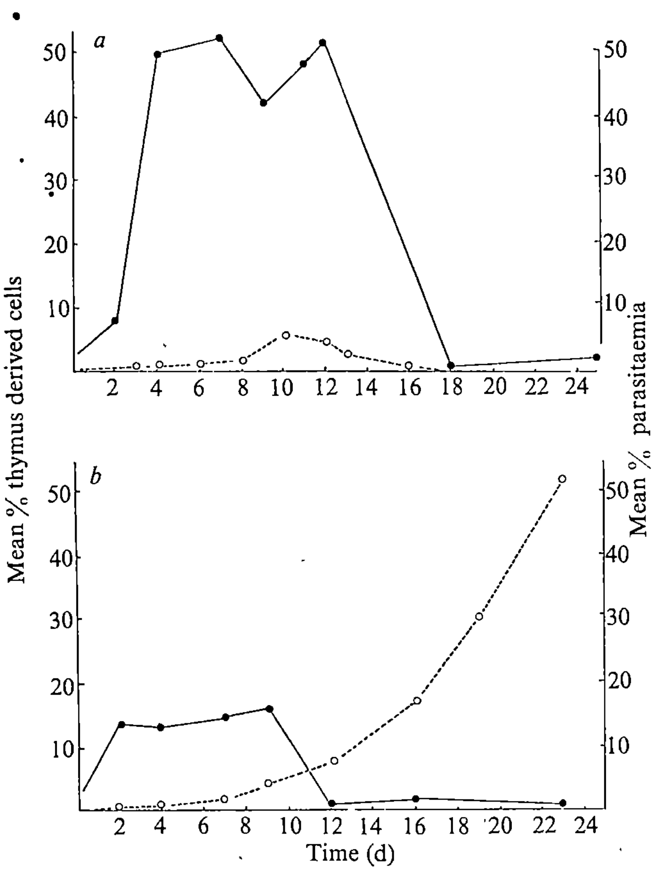


Fig. 1 Mean percentage of thymus graft derived cells (●) dividing in the spleen and mean percentage parasitaemias (○) in CBA/Lac–CBA/T_6T_6 mouse radiation chimaeras infected with 1×10^5 *P. yoelii* (*a*) or *P. berghei* (*b*) parasitised RBCs.

<1%), *P. yoelii* infected mice showed massive T-cell activation—53.1% of the dividing cells in the spleen being of certain thymic origin. This level of T-cell activation was maintained until day 13 Between days 13 and 18, during which time control of the infection seemed to have been achieved, as judged by the clearance of parasites from the peripheral blood, T-cell mitoses became much less frequent.

During the first 10 d of infection with *P. berghei*, parasitaemias were similar to those observed in mice infected with *P. yoelii*. Thereafter the parasitaemias increased steadily and proved fatal by day 24. The amount of T-cell activity elicited during a fatal *P. berghei* infection was proportionately much less than that observed in a resolving *P. yoelii* infection. Although by day 2 13.7% of the cells in mitosis were T cells (compared with 7.4% in *P. yoelii*), there was little change in the level of T-cell activation over the next 7 d and, from day 9 onwards, the response seemed to fail completely in spite of the continuously increasing parasite burden.

The precise functional significance of the T-cell mitosis observed in these infections is uncertain, but such mitosis could be indicative of the development of cytotoxic, helper,

Table 1 Fluorescent antibody titres in CBA mice infected with *P. yoelii* or *P. berghei*. Each determination was carried out on serum pooled from five mice using the indirect fluorescent antibody method for malaria parasites as described by Voller[10]

	Day of infection	Total Ig*	IgG_1†	IgG_2	IgM
P. yoelii	0	—	—	—	—
	7	32	—	32	256
	14	1,024	512	2,048	128
	21	1,024	128	512	256
	28	1,024	256	1,024	256
	35	1,024	256	512	128
P. berghei	0	—	—	—	—
	5	—	—	—	32
	9	32	—	32	128
	15	32	32	64	256
	18	64	128	128	64
	24	64	128	256	64

*Lyophilised swine anti-mouse globulin conjugated with fluorescein isothiocyanate (FITC) was purchased from Nordic Pharmaceuticals.

†Goat anti-mouse IgG_1, IgG_2 and IgM sera were obtained from Nutritional Biochemical Corporation, Cleveland, and were conjugated to FITC[11] isomere 1 (Nordic Pharmaceuticals).

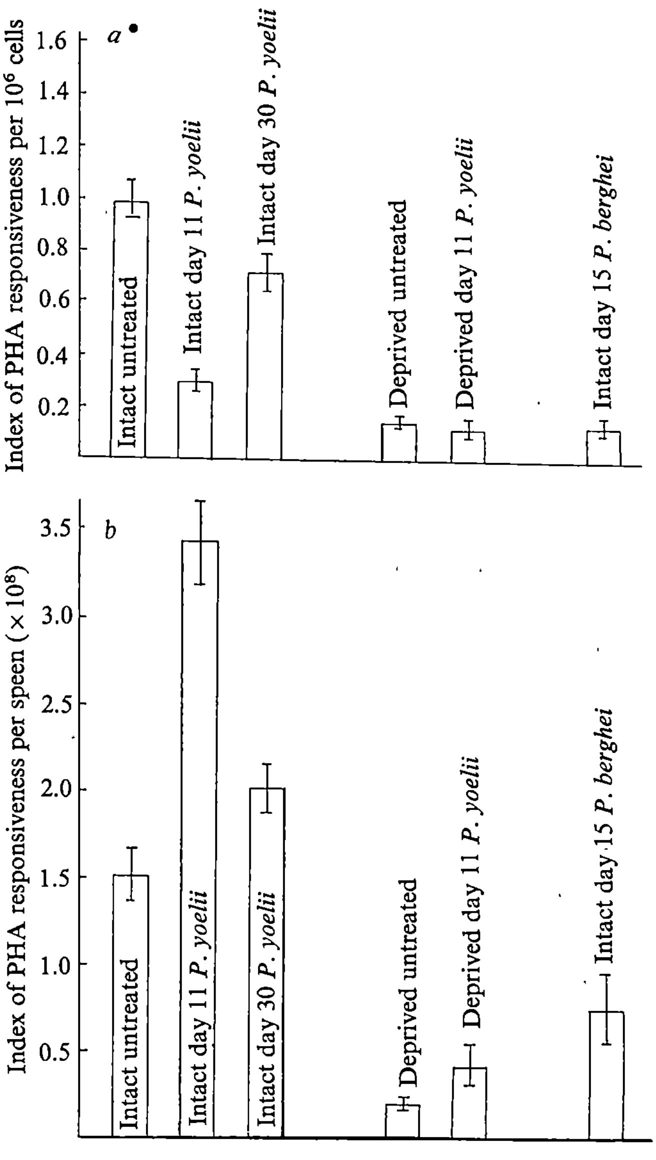
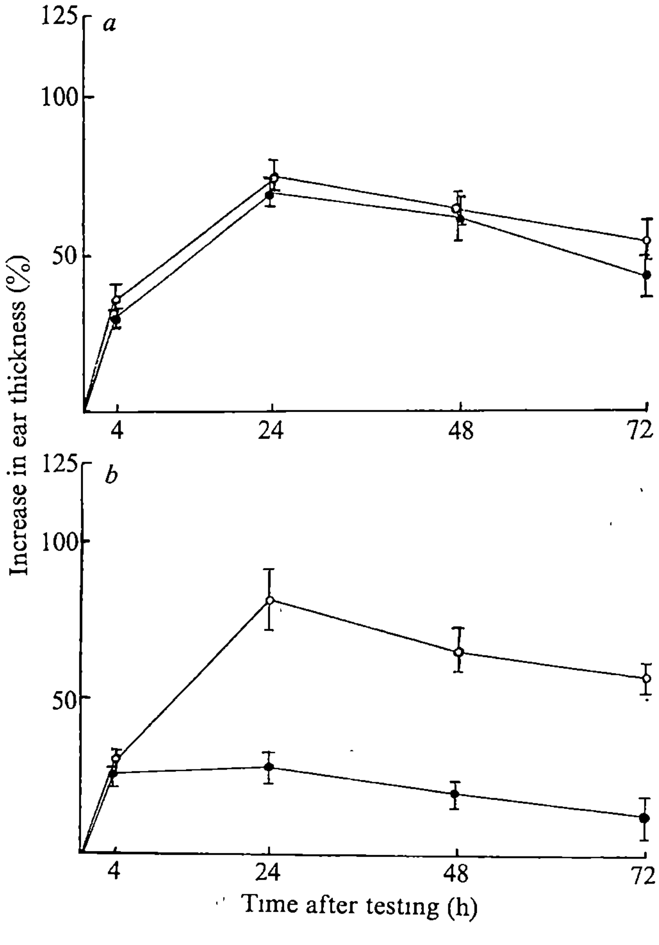


Fig. 2 The index of PHA responsiveness per 10^6 spleen cells (*a*) and the index of PHA responsiveness per spleen±1 s.e. (*b*) in normal and *P. yoelii* or *P. berghei* infected CBA mice. (Uninfected and *P. yoelii* infected T-cell deprived mice were included as additional controls) Each group consisted of five mice The method of quantitation used was that of Doenhoff[13] and is based on the cytological analysis of cells derived from CBA/Lac and CBA/T_6T_6 mice. (Cells of the T_6T_6 strain carry a pair of minute chromosomes by means of which they can be distinguished at metaphase.) A known number of cells from untreated CBA/T_6T_6 mice was mixed with an equal number of cells from CBA/Lac mice whose response to PHA was being determined. The mixture of the two cell types was cultured with PHA (Burroughs Wellcome) for 3 d, when, following metaphase arrest with colcemid, the cells were subjected to cytological analysis[9]. The ratio of the dividing cells from the two sources (Lac:T_6T_6) gave the relative concentration of PHA responsive cells, and this was designated the index of responsiveness per 10^6 cells. (A culture consisted of 1×10^6 cells.) To estimate the degree of responsiveness per spleen, the index per 10^6 cells was multiplied by the total number of nucleated cells in the spleen from which the cultured cells were derived

or some other T-cell-dependent effector activity such as lymphokine production. In *P. yoelii* infected mice, considerable T-cell activation occurred early in the infection at a time when only very low levels of antibody could be detected. (Fluorescent antibody levels are recorded in Table 1.) Once the parasitaemia and T-cell activity began to decline, peak levels of antibody were recorded and considerably elevated levels of these antibodies were maintained long after the T-cell response had died down It must be pointed out, however, that this temporal relationship between antibody production and the T-cell response could be due to excess circulating antibody being present only after regression of the blood parasitaemia. *P. berghei* infected mice produced significantly lower levels of antibody particularly in the IgG_1 and IgG_2 subclasses.

In a parallel series of experiments we examined two correlates of T-cell function—the response of spleen cells to the T-cell mitogen PHA[12] (Fig 2), and the response of infected animals to the skin sensitising agent oxazolone (Fig. 3) On day 11 of infection (the time of peak parasitaemia), *P. yoelii* infected mice showed a reduction in the proportion of PHA-responsive cells in the spleen. By this time, however, spleen cell numbers had increased from 1.53×10^8 on day 0 to 12.22×10^8. Thus, when the response was computed on a per spleen basis there seemed to be no absolute deficiency in PHA responsiveness—the response per spleen in the infected group being 3.42×10^8 compared with 1.51×10^8 in the controls. Thirty days after infection, both the proportion and total number of PHA-responsive cells in the spleen showed no significant difference from control values In contrast, *P. berghei* infected mice, when assayed on day 15 of infection, showed a severe reduction in the proportion of responsive cells comparable with that found in T-cell deprived mice and, even when calculated on a per spleen basis, the response was still significantly depressed. The ability to respond to oxazolone was also seriously impaired in *P. berghei* infected mice, whereas the response of *P. yoelii* infected mice, at the time tested, seemed normal.

Fig. 3 Mean percentage increase in ear thickness ± 1 s.e. in uninfected (○) and *P. yoelii* (*a*) or *P. berghei* (*b*) infected (●) CBA mice sensitised with oxazolone (five mice per group). Mice were sensitised (on day 8 of infection) by applying 0.1 ml of a 3% solution of oxazolone in alcohol to the skin of the abdomen and were tested 6 d after sensitisation by applying one drop of a 1% solution of oxazolone in olive oil on to both sides of the right and left ears[14]. Ear thickness was measured at 4, 24, 48 and 72 h after application of the test solution.



It can thus be seen that these two parasites exert very different effects on the T-cell pool; *P. yoelii* induces massive T-cell activation, the development of protective immunity and the responses to PHA and oxazolone seem normal. *P. berghei*, on the other hand, elicits limited T-cell activity—there being a failure of the T-cell response to the parasite and a generalised failure of T-cell function. Although transient depression of immune responsiveness to antigens such as SRBC, HGG (heat aggregated human γ globulin)[4] and tetanus toxoid[15] have been shown to occur *P. yoelii* infections—this is not necessarily due to suppression of T-cell function. Architectural changes in the spleen and consequent changes in cell relationships, dilution of responsive cells by non-responsive elements or some form of preemption[16] may be relevant to this temporary loss of immune responsiveness and may also contribute to the immuno-suppression in *P. berghei* infections.

Although it is not possible on the basis of the results presented here to establish definitely a causal relationship between T-cell activity and the development of immunity or the nonspecific suppression of T-cell function, these results suggest strongly a crucial role for T-cell activation and T-cell suppression in the pathogenesis of malaria.

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Genetics of natural resistance to herpesvirus infections in mice

INHERITED resistance to virus infections has been demonstrated in several murine systems[1-6]. Susceptibility to mouse hepatitis virus[3] and resistance to arbovirus[7] infections in mice are dominant traits associated with the capacity of macrophages from the susceptible strains to replicate virus. Resistance to Friend leukaemia virus- and Gross leukaemia virus-induced leukaemogenesis is determined by at least two genes, one closely linked to the immune response genes of the mouse[8]. Conversely, susceptibility to lymphocytic choriomeningitis (LCM) virus infections in mice has been associated with a gene closely linked to the immune response genes[9]. The latter seems to be associated with the cell-mediated immune response to LCM which causes tissue damage and leads to death. Cell-mediated immunity seems to be important in resistance against herpes simplex virus (HSV) infections[10-12]. Analysis of the aspects of cell-mediated immunity which might be involved in resistance in man is complicated by the uncontrollable factors associated with activation of latent herpesvirus infections. I have developed a mouse model which has shown that at least three genes are responsible for resistance to HSV. Although preliminary evidence suggests that resistance is immunologically mediated, resistance genes do not seem to be closely linked to the histocompatibility region of the mouse.

We used inbred strains of mice with high and low resistance. Since resistance to viral leukaemogenesis was demonstrated with an unusually virulent strain of virus[13] (Gross passage A[14]), we used an HSV-1 strain (2931) which is highly virulent in BALB/c mice. The strain was isolated from a human labial lesion and passaged ten times in Vero cells. The virus was then cloned to produce a homogeneous virus population, and thus a homogeneous response from the various mouse strains. Cloned virus was typed by the neutralising antibody test with antisera provided by Dr Donald Armstrong, Memorial Sloan–Kettering Cancer Center. A large pool was prepared by infecting Viro cells at a multiplicity of 1 plaque-forming-unit (PFU) per cell and incubating the cells for 24 h at 37 °C. Cells and debris were removed from the supernatant by centrifugation at 150*g* and the virus was stored at −80 °C in 1-ml samples. This preparation had a titre of 1×10^8 PFU ml^{-1} when titred at the beginning and end of the experiments. The same pool was used throughout.

Eleven strains of inbred mice were obtained from Jackson Laboratories and tested for their capacity to resist fatal HSV-1 infections. Adult (3–4-month-old) male mice were inoculated intraperitoneally with the stock pool of HSV-1. Six to twelve mice were inoculated with 0.1 ml containing 1–10^6 PFU per inoculum. Mice were observed for 21 d and an LD_{50} was determined for each strain by the Spearman–Karber method[15] (Table 1). Mice usually showed signs of encephalitis and died 5–10 d after inoculation. AKR, A, BALB/c, and C57BL/6 mice were provided by Dr E. A. Boyse of Memorial Sloan–Kettering Cancer Center to confirm that the resistance observed in the mice from Jackson Laboratories could be observed in the same strains from other sources. LD_{50}s were essentially the same and have been incorporated into Table 1. The eleven strains seem to fall into three categories: resistant, moderately susceptible and very susceptible. Most resistant mice survived inocula of 10^6 PFU of HSV-1, while LD_{50}s for moderately susceptible mice are in the range 200–1,000 PFU per inoculum. The LD_{50}s for very susceptible mice are in the range 10–70 PFU per mouse. Susceptible mice which survived the lower doses of virus were reinoculated 4–8 weeks later with 10^6 PFU of HSV-1. All survived reinoculation, indicating that they had developed resistance.

Table 1 Resistance of inbred mouse strains to HSV-1

Mouse strain	H_2	LD^{50}HSV-1 (P F.U.)
AKR (12)*	k	$10^{1,0}$
SWR (6)	q	$10^{1,0}$
A (12)	a	$10^{1,33}$
DBA (6)	d	$10^{1,83}$
CBA (6)	k	$10^{2,34}$
BALB/c (12)	d	$10^{2,34}$
C3H/He (10)	k	$10^{3,0}$†
C57BR (6)	k	$10^{3,0}$†
C57BL/6 (12)	b	$>10^6$
C57BL/Ks (6)	d	$>10^6$
C57BL/10 (20)	b	$>10^6$

Mice were inoculated intraperitoneally with 0.1 ml of tenfold dilutions of stock HSV-1 pool and observed for 21 d. Mice died with signs of encephalitis usually 5–10 d after inoculation. LD_{50} was determined by the Spearman–Karber method[15].

*No. test per dilution.

†Titrations not clear cut Two C3H and four C57BR mice survived at lower dilutions. Others died as if to yield the LD_{50} noted. In a second experiment, 20 of each strain were inoculated with 10^6 PFU. Sixty per cent of the C3H/Hc and none of the C57BR mice died.

To determine whether this pattern of resistance and susceptibility was the same for other HSV-1 strains, similar experiments were carried out with three further wild strains of HSV-1 as well as with HSV-1 strains obtained from Dr Fred Rapp (through Michael Bernhardt), Dr Barry Bloom and from the American Type Culture Collection (ATCC). The MacIntyre strain (VR_3) is the prototype HSV-1 strain held by the ATCC and has been used in studies of resistance by Nahmias *et al.*[10] and Stevens and Cook[15]. All strains of HSV-1 have the same relative virulence in C57BL/6, BALB/c and A/J mice, as observed for HSV-1 (strain 2931) (Table 2). The exception was strain HEFM[16] which seems to have lost its virulence for mice. HSV-1 (strain 2931) seemed to be more virulent than the other strains and continues to be used for this study.

Table 2 Virulence of HSV-1 strains

Strain (inoculum)	% killed by HSV-1 A/J	 BALB/c	 C57BL/6
2931 (1×10^4 PFU)	100	100	0
A.P. (2.7×10^4 PFU)	100	100	0
1895 (1×10^5 PFU)	100	60	0
MacIntyre (5×10^4 PFU)	100	60	0
Patton (1.67×10^3 PFU)	100	20	0
M D (1.3×10^4 PFU)	60	20	0
HEFM (2.2×10^6 PFU)	0	0	0

Five mice of each prototype strain were inoculated intraperitoneally with 0.1 ml of the seven HSV-1 strains listed. The amounts of virus inoculated is in parentheses. All mice were observed for 21 d and the percentage killed by each virus strain was calculated.

To determine whether resistance is dominant, crosses were produced between resistant and very susceptible mice ($(C57BL/6 \times A)F_1$) and between resistant and moderately susceptible mice ($(C57BL/6 \times BALB/c)F_1$). Twenty of each of these F_1 animals were inoculated with 10^6 PFU of HSV-1 (Table 3). Ninety per cent of the $(C57BL/6 \times A)F_1$ mice and 100% of the $(C57BL/6 \times BALB/c)F_1$ mice survived, indicating that resistance was dominant.

Further crosses were made to determine the number of genes involved in resistance (Table 3). $(C57BL/6 \times A)F_1$ mice were backcrossed to A/J strain mice. Forty-five adult progeny were inoculated with 10^6 PFU of HSV-1 and 34 (75.5%) of these died. If one dominant gene were involved, assuming parental homozygosity, 50% of the mice would have been susceptible. These data indicate that at least two genes are involved in resistance. $(C57BL/6 \times BALB/c)F_1$ mice were backcrossed to BALB/c mice and 37 of these were inoculated with 10^6 PFU of virus. Only three (8%) of these died. This makes the genetic evaluation more difficult and may indicate that four or more genes are involved in resistance. Specific interaction between genes would also make an evaluation of this type difficult.

Resistance to some murine virus infections is determined by

Table 3 Resistance of crosses inoculated with 10^6 PFU HSV-1

C57BL/6 (40*)	90% live
BALB/c (20)	0% live
A (20)	0% live
(C57BL/6 × A)F_1 (20)	90% live
(C57BL/6 × BALB/c)F_1 (20)	100% live
(C57BL/6 × A)F_1 backcrossed to A (45)	25% live
(C57BL/6 × BALB/c)F_1 backcrossed to BALB/c (37)	92% live

Mice were inoculated intraperitoneally with 0.1 ml HSV-1 containing 10^6 PFU. Percentage of mice surviving 21 d was calculated. *No. of mice per test.

genes linked to the major histocompatibility complex of the mouse. Our preliminary results indicate that resistance to HSV-1 is not associated with a particular histocompatibility (H-2) allele. Resistant mouse strains were of H-2^b and H-2^d types. One susceptible and one moderately susceptible strain was also found to be of the H-2^d type. H-2H and H-2I mice were obtained from Dr Boyse to study this question further. These mice are crosses between C57BL/6 (resistant) and A/J strain (very susceptible) mice in which there is a crossover between the K and D ends of the H-2 region. The H-2H strain carries the K (Ir) region of the H-2I (A/J strain) and the D region of the H-2^b(C57BL/6), and the H-2I carries the reciprocal H-2 crossover. If the K region of the A/J strain is associated with susceptibility to HSV-1, as has been shown for susceptibility to viral leukaemogenesis, the H-2H should be susceptible. They were resistant (86%) to 10^3 PFU of HSV-1. Thus either the H-2 region of the A/J strain mice is not associated with susceptibility to HSV-1 or it is being masked by another resistance gene.

We also used congenic mice obtained from Dr Boyse. Eleven C57BL/6 mice into which the H-2^k allele of the AKR strain had been introduced (B6/H-2^k) and five AKR mice into which the H-2^b allele of C57BL/6 had been introduced (AKR/H-2^b) were inoculated with 10^3 PFU of HSV-1. The B6/H-2^k mice were resistant while the AKR/H-2^b mice were susceptible, indicating that the H-2^k allele did not confer susceptibility on C57BL/6 mice and the H-2^b allele did not confer resistance on AKR mice. These studies must be confirmed with more mice and a broader range of inocula. More definitive evidence for an association of H-2 allele with resistance will come from H-2 typing of resistant and susceptible backcross progeny.

Although genes governing resistance to HSV-1 do not seem to be closely linked to the H-2 complex, and thus the immune response genes of the mouse, preliminary evidence indicates that resistance is not a property of the susceptibility of structural cells and is probably mediated immunologically. Embryo fibroblast cultures were prepared from A/J, BALB/c and C57BL/6 mice and inoculated with HSV-1. Virus replicated to the same titre in each monolayer, indicating that resistance was not mediated by the inability of HSV-1 to replicate in structural cells of resistant mice. Preliminary lymphocyte transformation studies with splenic lymphocytes of mice inoculated 5–6 d earlier with HSV-1 indicate that resistant and moderately susceptible mice were capable of a vigorous response whereas very susceptible mice did not respond above control level (C.L. and R. O'Reilly, unpublished). F_1 crosses between resistant and very susceptible mice were also capable of a strong lymphocyte transformation response to HSV-1, indicating that this characteristic is inherited as a dominant trait.

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Prevention of rabies by vitamin C

MURATA[1] reported on the virucidal activity of vitamin C and stated that vitamin C prevents various virus diseases. Clinical trials by F. Morishige (unpublished) showed that the incidence of transfusion hepatitis was decreased drastically when vitamin C was injected (3–7 g d^{-1}) for 1–2 weeks after blood transfusion, and Murata has pointed out that vitamin C is effective in the therapy of measles and mumps[1]. I have now found that it also helps to prevent rabies.

Guinea pigs, fixed rabies virus and vitamin C (Lek, Ljubljana) were used in the experiments. Five groups of ten test animals and ten control animals were inoculated intramuscularly with 1 ml of a 10% emulsion of rabbit brain containing LD_{50}–LD_{90} of the infectious fixed rabies virus. Treatment of the test animals was initiated 6 h after inoculation, and vitamin C was injected intramuscularly twice a day for 7 d; each dose contained 100 mg per kg body weight. Control animals were injected with saline. The animals were observed for 14 d. Most of those that succumbed to the inoculation became paralysed on the sixth or seventh day. Some became paralysed on the fifth day, and a few others not until the eighth or tenth day.

The frequencies of deaths from rabies in the five groups treated with vitamin C were: 5/10, 2/9, 6/9, 1/10, 3/10. Of the control animals, however, 9/10, 6/10, 9/10, 6/10 and 5/10 died (Table 1). Thus 17/48 (35.42%) of the treated animals and 35/50 (70%) of the controls died of rabies. The χ^2 value for these frequencies is 11.107, so that the difference between the treated and control groups is statistically significant ($P > 0.01$). My results therefore justify the conclusion that vitamin C is effective in rabies prevention. It had no therapeutic effect, however, for in animals which developed paralysis, continued treatment was always ineffective.

Table 1 Frequencies of deaths in guinea pigs inoculated with fixed rabies virus and treated with vitamin C and in the control animals.

Treatment	Deaths	Survivors	Σf
With vitamin C	17	31	48
Controls	35	15	50
Σf	52	46	98

In preliminary experiments, not reported here, lower doses of vitamin C (25 and 50 mg kg^{-1}) were tried in a small group of guinea pigs. These doses were less effective than the high dose used in the reported experiments. To establish the optimal dose of vitamin C, however, exact dose–response experiments will have to be done on sufficiently large groups of animals.

To exclude the possibility that rabbit brain itself contributed in any way towards paralysis and mortality of guinea pigs, another group of 30 guinea pigs was inoculated with 1 ml of a

10% emulsion of rabbit brain which was free of virus. All animals survived an observation period of 14 d without any signs of disease.

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Increased insulin binding capacity of liver membranes from diabetic Chinese hamsters

THE first evidence for an abnormality in insulin receptor capacity in pathological states was provided by Kahn *et al.*[1], who found that liver plasma membranes from the obese hyperglycaemic mouse bound only a fraction of insulin compared with membranes from lean littermates. A similar observation was made with lymphocytes from obese insulin-resistant diabetic patients[2]. In lymphocyte cultures the insulin concentration in the medium seemed to determine the binding capacity of the cells[3]. These results suggested an inverse relationship between the number of receptors and the surrounding concentration of insulin, regardless of the tissue sensitivity to the hormone. We have now found greater than normal binding capacity in the diabetic Chinese hamster, which has genetic insulin deficiency, in many ways resembling juvenile diabetes[4].

Table 1 Characteristics of control and diabetic groups and the respective plasma membrane preparations (10 animals in each group)

	Control	Diabetic
Body weight (g)	36 9±0.3	34.8±0.8
Liver weight (mg)	1.45±0.05	1.71±0.04
Blood sugar (mg per 100 ml)	130±6	468±59
5′-Nucleotidase (μmol $\text{mg}^{-1}\times$min)	0.36	0.33
Mg^{2+}-ATPase (nmol $\text{mg}^{-1}\times$min)	179 2	168.8
Recovery of membrane protein (mg per g wet weight)	1.03	0.94

5′-Nucleotidase was measured as recommended by Solyom *et al*[6] Mg^{2+}-ATPase was assayed by measuring the release of ^{32}P-orthophosphate from γ-^{32}P-ATP (ref 7).

Plasma membranes were prepared by the method of Neville[5] from diabetic Chinese hamsters aged 4–5 months and from control animals of comparable age and weight (Table 1). ^{125}I-insulin binding was assayed after incubation for 20 min at 25 °C, membrane-bound hormone was separated from free hormone by centrifugation through silicon oil (W K., R.R , A. Zynamon and K.D.H., in preparation). Figure 1 is a Scatchard plot from a representative experiment. As previously observed in rats, the points fit two curves corresponding to high affinity–low capacity (site I) and low affinity–high capacity (site II) binding sites[8]. The constants derived from this figure are—for site I: control, 4.2×10^{-13}, diabetic, 9.0×10^{-13} mol per mg protein; for site II: control, 1.9×10^{-12}, diabetic, 4.0×10^{-12} mol per mg protein The corresponding affinity constants are approximately 10^{-9} for site I, and 10^{-8} mol l^{-1} for site II, in both groups. Recovery of membrane protein and enzyme markers was similar for both groups (Table 1). Figure 1 shows that the binding capacity for both classes of binding sites (intercept at abscissa) per mg protein is considerably higher in the diabetic animals, whereas there is no apparent change in affinity (represented by the slope). Although other interpretations of nonlinear Scatchard plots, such as negative cooperativity[9], polymerisation of the hormone[10] or heterogeneity of the labelled hormone[11] are possible, the essential observation—of an abnormally high binding capacity in membranes from diabetic hamster—remains the same.

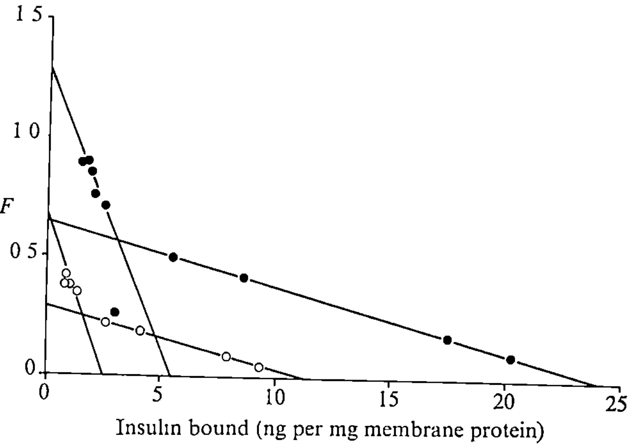

Fig. 1 Scatchard plot of insulin–membrane interaction in two representative preparations from 10 control (○) and 10 diabetic (●) animals. Incubation was performed at 25 °C for 20 min with 0.2 mg of membrane protein per ml and insulin concentrations between 4 and 400 ng ml^{-1}. Insulin degradation was 1.3% in these conditions. Nonspecific binding, that is labelled hormone not displaced by 1 U ml^{-1} has been subtracted.

Severely diabetic Chinese hamsters have abnormally low plasma and pancreatic insulin, a low insulin response to a glucose load and, consequently, low glucose tolerance[4]. Together with a tendency for ketosis[4], these features resemble juvenile diabetes in man, and the diabetic Chinese hamster provides an interesting model for this type of the disease. Apparently the liver adapts to the decreased hormone concentration by raising the number of receptors in the plasma membrane, whereas, conversely, in the hyperinsulinaemic C57BL ob/ob mouse[1] as well as in the KK-mouse[12], the total receptor capacity is lower than normal. Our data therefore support the hypothesis that the hormone level controls the number of receptor sites in the liver cell

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Synaptic coupling into the production and storage of a neuronal memory trace

An adequate model for the nature of the memory trace at the neuronal level should provide for a sequence of physiological mechanisms which would include 'read-in' of the neuronal change, 'storage' processes that impart an enduring quality, and 'read-out' of the change in the form of an altered neuronal response to an appropriate neural input. The framework for such a neuronal model seems to be provided by the features of a novel heterosynaptic interaction in a mammalian sympathetic ganglion It had been found previously that one synaptic transmitter (the catecholamine dopamine, DA) could produce a specific and enduring enhancement, lasting for at least several hours, of the subsequent responses to another synaptic transmitter (acetylcholine, ACh)[1]. We have now found that the retention of this DA-induced change can be disrupted by cyclic GMP in a strikingly time-dependent manner that is similar, in principle, to the well known disruptability feature of the memory storage process[2,3]. Additional evidence indicates that the production and storage of the enduring change initiated by DA are mediated intraneuronally by cyclic AMP. We present here a scheme which outlines the cellular pathways postulated to carry out the sequence of events from 'read-in' through to 'read-out'.

The rabbit superior cervical ganglion was studied *in vitro* at ~ 22 °C, in a superfusion chamber with a high resistance sucrose-gap along a section of postganglionic nerve between the recording leads[1]. The section containing the ganglion proper had a volume of 0.3 ml and was perfused continuously with Krebs–Ringer solution at about 1 ml min^{-1}. The test response consisted of the slow depolarisation of the ganglion cells produced by the muscarinic agonist acetyl-β-methylcholine (MCh)[4]. The variable complicating factor of a hyperpolarising component in the response to such cholinergic agonists, which is due to the action of DA released from an interneurone, was eliminated by a pretreatment with the muscarinic agent bethanechol; the latter depletes the ganglion of its functionally-releasable DA[5]. To observe the DA-modulatory action, the slow depolarising response to a test dose of MCh is obtained before and at various times after a brief superfusion with a single supramaximal dose of DA (for example, Fig. 1, compare tracings *a, b* with *c, d*). Enhancement of the cholinergic responses can persist unabated for the duration of the ganglion's survival *in vitro*, that is more than for 3 h.

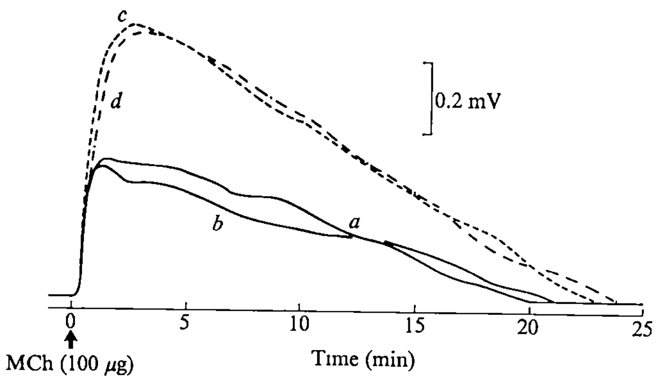

Fig. 1 Modulatory enhancement of methacholine (MCh)-depolarisation after dopamine (DA), and the absence of any effect on this when cyclic GMP was applied 30 min after DA. Each tracing is the slow depolarising response to a single injection of MCh (100 µg). *a* and *b*, Controls before DA *c*, MCh response at 60 min after a single injection of DA (50 µg), with an interposed addition of cyclic GMP (50 µM for 8 min) which began at 30 min after DA. *d*, MCh response at 60 min after a second dose of DA, with no interposed treatment by cyclic GMP.

The usual DA-produced enhancement of the MCh-depolarisations could be prevented if dibutyryl cyclic GMP (50 µM) was temporarily added (for 8 min) to the superfusing fluid. Disruption of the expected modulatory effect could be complete even when the onset of cyclic GMP treatment was delayed for up to 4 min after the DA injection (Fig. 2, compare tracings *a, b* with *c*). A succeeding, second exposure to DA, which was not followed by treatment with cyclic GMP, could still produce the usual later enhancement of MCh-depolarisations (Fig. 2, compare tracing *c* with *d, e*). Guanosine 5′-monophosphate (non-cyclic GMP) could produce some small disruptive effects, guanosine, dibutyryl cyclic AMP and adenosine 5′-monophosphate (non-cyclic AMP) had no disruptive action Additionally, an 8-min treatment with Ringer containing 10 mM K$^+$ simulated the depolarisation that is often elicited by the dibutyryl cyclic GMP itself, but it had no disruptive effect on the DA-modulatory action.

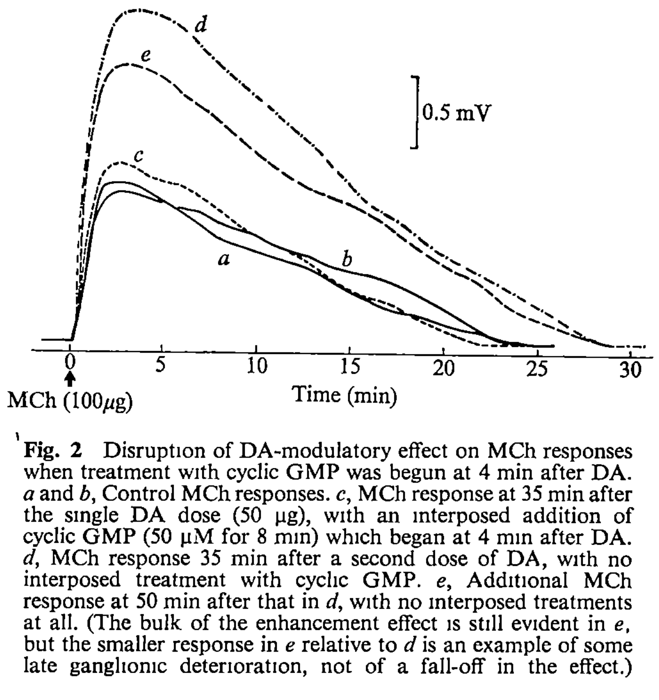

Fig. 2 Disruption of DA-modulatory effect on MCh responses when treatment with cyclic GMP was begun at 4 min after DA. *a* and *b*, Control MCh responses. *c*, MCh response at 35 min after the single DA dose (50 µg), with an interposed addition of cyclic GMP (50 µM for 8 min) which began at 4 min after DA. *d*, MCh response 35 min after a second dose of DA, with no interposed treatment with cyclic GMP. *e*, Additional MCh response at 50 min after that in *d*, with no interposed treatments at all. (The bulk of the enhancement effect is still evident in *e*, but the smaller response in *e* relative to *d* is an example of some late ganglionic deterioration, not of a fall-off in the effect.)

When treatment with cyclic GMP was delayed for periods longer than 4 min after DA, progressively lesser disruptions occurred. With delays of greater than 10–15 min, there was no disruption; that is, retention of the modulatory effect of DA was then unaffected by cyclic GMP (Fig. 1). An initial manifestation of the modulatory enhancing action, which could be observed when DA was injected during a continuous superfusion of MCh (0 5 µM), was also unaffected by the presence of cyclic GMP.

These features of the actions by cyclic GMP suggest that, after the initial modulating change is induced by DA, a 'storage' process develops which leads to a more durable form of modulatory change (see Fig. 3); in this view, the storage process (but not the initial or the durable products) can be antagonised or disrupted by cyclic GMP. On the basis of further evidence (below) we also propose that cyclic AMP is the intracellular mediator for processes I and II in Fig 3, that is, for producing the DA-enhancing action and its 'storage'. This function would be additional to the already proposed role[6–8] for cyclic AMP as a mediator of a direct inhibitory action of DA, which had also been previously described[1,4].

The ability of cyclic AMP to produce an enduring enhancement of the depolarising response to MCh was demonstrated by substituting a treatment with dibutyryl cyclic AMP (1 mM for 6–10 min) for the dose of DA, in

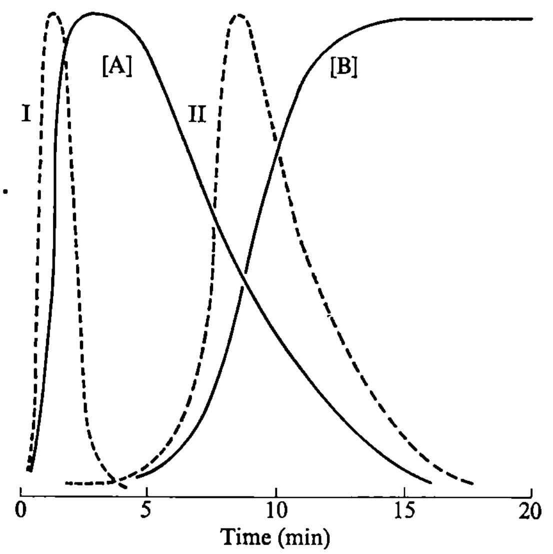

Fig. 3 Time curves for processes and cell products proposed as components in the DA-modulatory action. Process I would involve reactions that lead to the molecular changes which are responsible for the initial enhancement of the slow depolarising response to ACh (the latter is equivalent to the slow excitatory postsynaptic potential, or s-e.p.s.p.). The nature and site of these DA-induced molecular changes, in the unique sequence of events that begins at the ACh-muscarinic receptor and ends with the s-e.p.s.p. response[4], are not yet known, but we may refer to the relevant altered molecular state [A] as the 'product' of process I. Without further processing, [A] would gradually decay over a period of some minutes (certainly less than 30 min), and the responsiveness of the neurone to ACh (or MCh) would revert to the resting control level that existed before the DA modulating action. Cyclic GMP seems to have no effect on process I, or on product [A] and its effectiveness. Process II represents a storage-type of reaction which converts [A], or a separate precursor of [A], into the more durable altered state [B].

experiments similar to those in Figs 1 and 2. The enhancement produced by cyclic AMP could occlude fully the production of any further enhancement by a subsequent dose of DA; and it could also be disrupted by cyclic GMP in a time-dependent manner. It may be argued that the similarity of actions by cyclic AMP and DA is simply due to DA in both cases, with cyclic AMP producing a presynaptic release of DA (a possibility raised by reports for certain other cases[9]). This possibility seems, however, to be excluded here. (1) As noted, the ganglia were almost completely depleted of their functionally-releasable DA by a suitable pretreatment[4,5]. (2) Treatment of a ganglion for 50 min with monobutyryl cyclic AMP (in the presence of theophylline and α-methyl-p-tyrosine) produced no evidence of depletion of functional DA, in contrast to that seen with a briefer bethanechol treatment. The additional important requirement, for a proposal that cyclic AMP mediates the DA-modulatory action, is that DA (or a suitable input of preganglionic nerve impulses) be capable of eliciting an adequate increase in cyclic AMP in the ganglion cells. This requirement has already been met adequately by previous findings[7,8,10].

The scheme presented in Fig. 4 provides a theoretical outline of the full sequence of the physiological actions involved in producing, storing, and 'reading-out' the enduring modulatory effects on the s-e.p.s.p. responses to cholinergic inputs. We do not exclude the possibility that

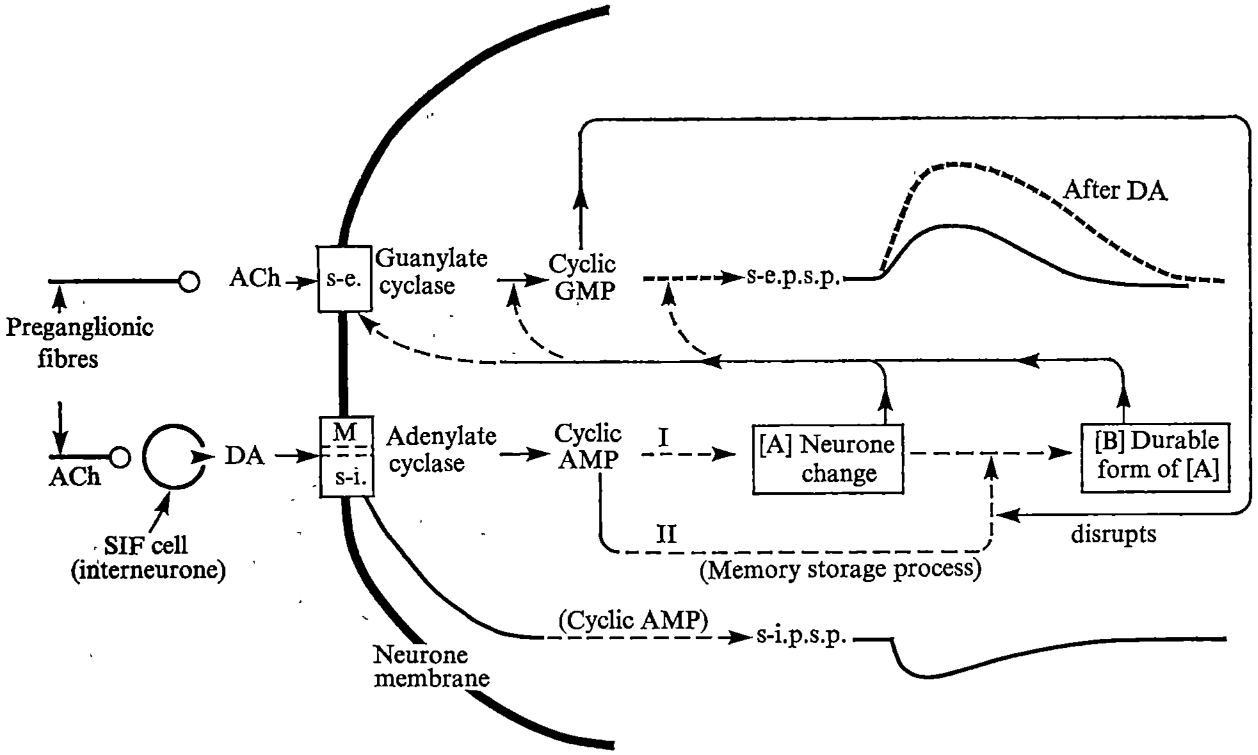

Fig. 4 Schema of pathways for synaptic inputs and intracellular mediators involved in the responses to DA and ACh, for a principal neurone (or ganglion cell) in the rabbit superior cervical ganglion. The s-e.p.s.p. is a slow depolarising change in the membrane potential in response to ACh acting at a muscarinic type of membrane receptor (s-e.); it is represented both before and after (dashed line) its enhancement by a modulatory action of DA. The s-i.p.s p. is a slow hyperpolarising change in membrane potential produced by a direct action of DA. DA is released by a special type of interneurone[5] (the small intensely-fluorescent, SIF, cell as seen with the Falck–Hillarp technique), in response to preganglionic impulses or to an applied muscarinic agonist of ACh[4]. The two postsynaptic actions of DA[1] are designated as M for the modulatory one and s-i. for the hyperpolarising response or s-i.p.s.p.; the actions could be mediated by a single or by two separate membrane receptors. Persisting enhancement of the s-e.p.s.p. response after exposure to DA would be somehow dependent on neuronal change [A] and its more durable form [B]. The question of the precise sites (in the sequence of reactions leading to the s-e.p.s.p. response) at which the modulating neuronal change is operative, is at present being investigated and is represented by a set of dashed arrows. (The proposals for a guanylate cyclase–cyclic GMP role in mediation of s-e.p.s.p. response, and for cyclic AMP as mediator of s-i.p.s p., were made previously[5–8,12].)

cyclic AMP may mediate the direct inhibitory action of DA[6–8], in addition to the enduring modulatory action by DA. On the other hand, the present evidence that cyclic AMP can mediate the DA-modulatory action suggests that this role could be an important one for the DA- (and possibly the noradrenaline-) coupled increases of cyclic AMP in various brain neurones[11].

The DA-modulating action in the sympathetic ganglion provides a model in which the development of a shorter term memory, a memory storage process, and the production and retention of a longer term memory trace, are all accomplished within the same single neurone and are all initially set into motion by the same intracellular messenger (cyclic AMP) following a single brief type of synaptic input. Repetitive synaptic actions fed in by reverberating circuits of nerve cells have been postulated to underlie short term memory and memory storage[2,3], but these are neither necessary nor available in the ganglion. In the present model, a heterosynaptic interaction takes place between two types of synaptic inputs to the same neurone; the memory trace is initiated by a brief (dopaminergic) input in one synaptic line, while the 'read-out' of the memory consists simply in the enhanced ability of the postsynaptic unit to produce its specific response to another (cholinergic) synaptic input. This arrangement provides for a 'learned' change in the response to one input as a result of an 'experience' previously carried by way of another input.

This proposed model does not in itself provide for the specificity of content of a given memory. Some prevalent hypotheses have attempted to provide for such specificity by postulating a specific memory trace[13], but they have suffered from an inadequacy in providing for both the 'read-in' and 'read-out' of the putatively encoded information. Perhaps the specific nature of a given engram could be sought outside the confines of the individual cell in group patternings, but based on the acquisition, by some individual neurones in these groups, of a memory trace that is itself not so specific. The individual neuronal memory portion of such an engram would then acquire a more general character common to many neurones. The view that our proposed model or variants of it may in fact have some general significance receives some support from indirect lines of evidence; the latter have added monoaminergic[14] to the cholinergic pathways already known to widely innervate the forebrain, and have indicated that cholinergic[13] and various monoaminergic transmitters[15] may have roles in long lasting functions of the central nervous system, including those in learning and memory.

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Molecular anisotropy of the early *Drosophila* embryo

IT is generally accepted that cellular differentiation depends chiefly on differential gene activity[1]. The model of differential gene activity implies the existence of different inducers/repressors activating/repressing different genes. Indirect evidence for such molecules in *Drosophila* comes from experiments of Illmensee and Mahowald[2] who showed that the information for primordial germ cell determination is in the cytoplasm of the posterior pole of the egg and that when this cytoplasm is transferred to the anterior pole so is the information. Their experiments suggest that genes are regulated not only in time but also in space, possibly by molecules distributed unequally in the developing system[3]. We have analysed *Drosophila* eggs for such molecules.

Kock and Heinig[4] and Nuneman and Moser[5] first approached the problem of unequally distributed molecules in insect eggs by microelectrophoresis and immunoelectrophoresis of parts of the egg of *Achaeta domestica*. They found quantitative differences between the anterior and posterior halves of the egg which probably depend on the unequal distribution of yolk platelets. We have approached this problem in *Drosophila melanogaster* by first developing a technique for producing extracts of different parts of the egg and then producing antisera against these extracts.

Eggs were collected on agar plates seeded with fresh bakers' yeast from population cages containing about 10,000 flies. Eggs (500–700) were oriented vertically on a flat surface with the posterior poles lying in the same plane. When the eggs had reached the desired developmental stage they were frozen in a glass frame. The dimensions of

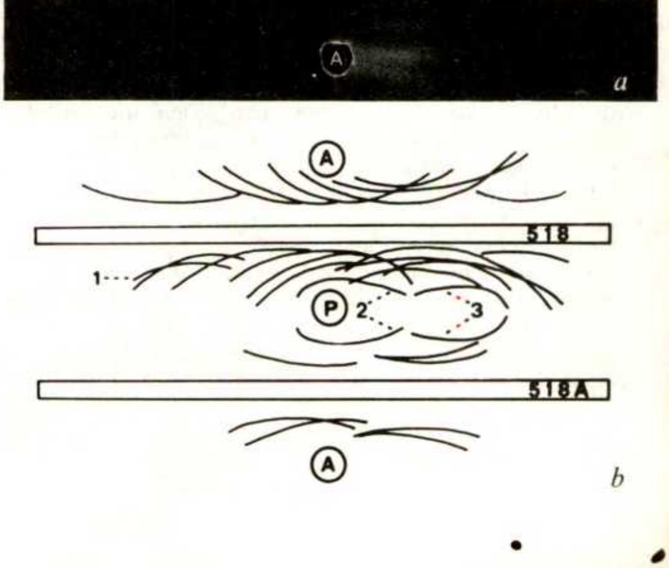


Fig. 1 *a*, Immunoelectrophoresis of 20% posterior (P) and 80% anterior (A) pole extracts. Protein concentration 40 mg ml^{-1}. Bands 1, 2 and 3 were found only in P. Antiserum 518 and antiserum 518A which was absorbed with A. Note that bands 2 and 3 remain unchanged with the absorbed antiserum while other bands either disappear or become weaker. *b*, Tracing of immunoelectrophoresis plate.

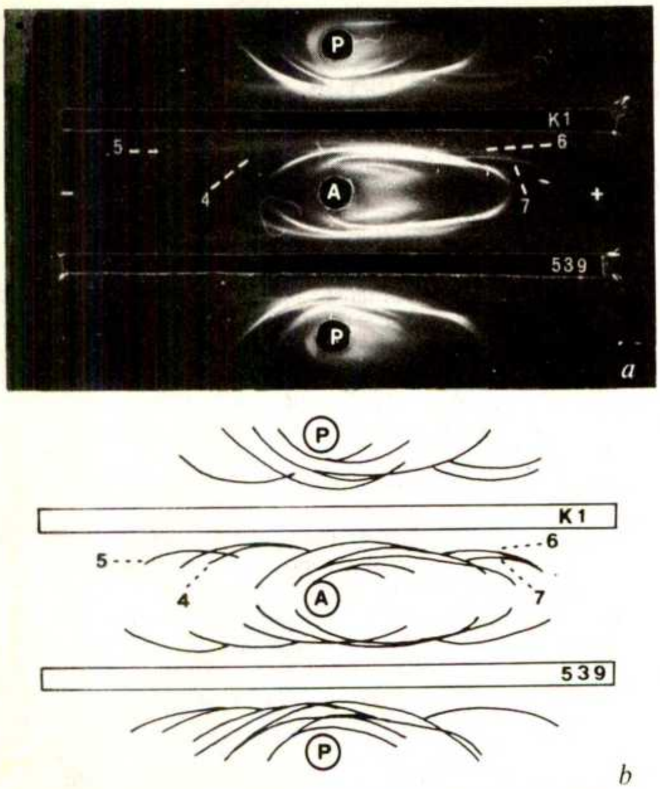


Fig. 2 *a*, Immunoelectrophoresis of 30% anterior (A) and 70% posterior (P) pole extracts. Protein concentration 40 mg ml^{-1}. Band 5 was detected only in A. Bands 4, 6 and 7 show a significant quantitative variation between A and P where they were more concentrated. Antiserum Kl and antiserum 539 also anti-0–4 h embryo. *b*, Tracing of immunoelectrophoresis plate.

the frames were such as to allow different proportions of the eggs to protrude above the surface. The protruding portions of the eggs were cut off in a cryostat microtome and the two parts of the eggs collected in different tubes. By adjusting the dimensions of the frame, the posterior 20%, 30%, 40% and so on, as estimated by the relative protein concentration[6], could be cut off.

The preparation of antisera and other immunological techniques were as reported previously[7]. The extracts were prepared by sonicating the cut embryos. Nine different antisera were used and only three of these showed differences in the antigen patterns of the extracts from different parts of the egg.

Antiserum 518 (0–4-h embryo extract, 30–40% ammonium sulphate fraction) showed antigens present in the posterior pole but not in the anterior pole represented by precipitin arcs 1, 2 and 3 (Fig. 1). These antigens were detected in the posterior 20% of the egg. If larger portions of the posterior part were taken, however, the weaker antigens could not be detected and the stronger ones became weaker, as would be expected if the antigens were located in the extreme posterior cytoplasm and were diluted with anterior cytoplasm lacking them. When the antiserum was absorbed with an extract of the anterior 80% of the egg and used to analyse extracts of the posterior 20%, arcs 2 and 3, identified as being in the posterior part of the egg, were unchanged on immunoelectrophoresis plates while the other arcs disappeared or were reduced because of the removal of the antibodies by absorption (Fig. 1).

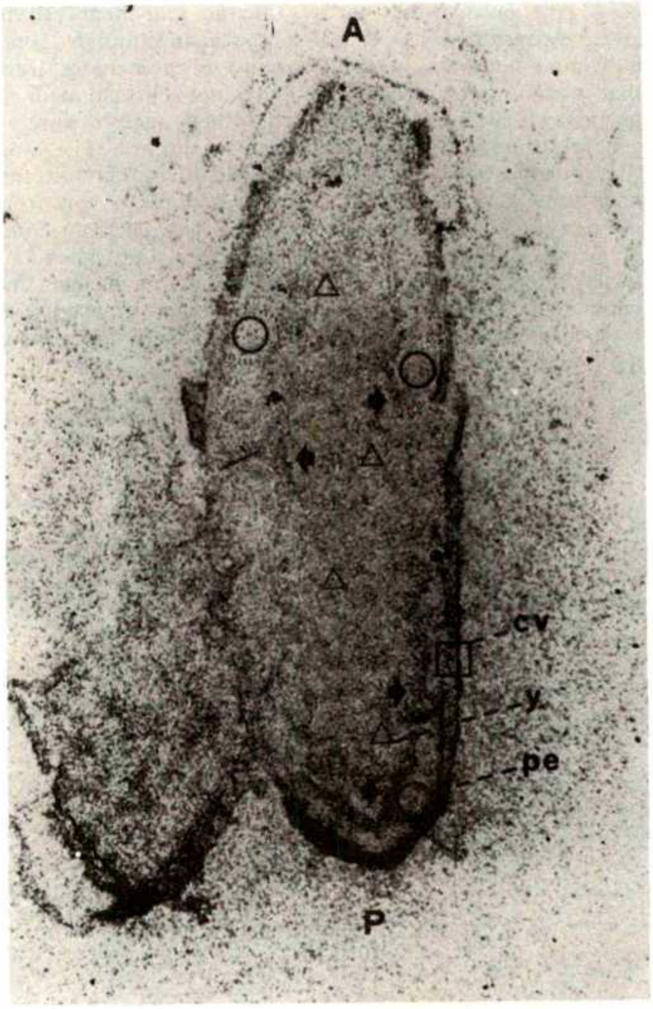
A second antigen showing unequal distribution in the egg was detected with antiserum Kl (0–4-h embryos). Extracts of the anterior 30% and 70% of the egg were compared with extracts of the posterior 70% and 30% respectively. Antigen 5 (Fig. 2) was limited to the anterior 30% of the egg. This antiserum also shows significant quantitative differences.

Antiserum 547 was prepared against an extract of the posterior 30% of the egg and was used to compare extracts of the anterior 30% and 70% of the egg with extracts of the posterior 70% and 30% respectively. One antigen was found in the posterior 30% fraction and not in the anterior 30% or 70% fractions. A second antigen was always associated with the larger portion of the egg irrespective of whether it was the anterior or posterior 70%. We suggest that this antigen is found in the central cytoplasm (including the yolk) and is detected in those extracts with the highest percentage of the cytoplasm, that is, the extracts of the larger part of the egg.

In order to test our proposals that antiserum 547 detected antigens associated with the posterior egg cytoplasm and with the central cytoplasm, as well as more general antigens, we sectioned frozen eggs with a cryostat microtome. The sections were flooded and incubated with antiserum 547, washed with saline and treated with ^{125}I-labelled horse anti-rabbit immunoglobulin. Autoradiographs of the washed slides show most of the radioactivity in the posterior pole of the egg and peripherally in the central cytoplasm at the boundary between the periplasm and the yolk mass thus confirming our prediction of the distribution of antigens detected by this antiserum (Fig. 3).

Although no technique allows us to say that a molecule is not in a certain part of the egg, these results demonstrate

Fig. 3 Autoradiograph of a section of 120-min-old egg treated with antiserum 547 followed, after washing, by treatment with ^{125}I-labelled horse anti-rabbit IgG. Treatment of similar sections with non-immune serum 547 and horse antibody or with horse antibody alone showed only nonspecific association of radioactivity with the chorion and a low overall background. cv, Chorion and vitelline membrane; pe, periplasm; y, yolky cytoplasm; A, anterior pole; P, posterior pole; arrows, boundary line between the periplasm and the yolky cytoplasm.



that some antigens at least are not distributed homogeneously in the egg cytoplasm. This result is all the more interesting as the antigens studied on double diffusion plates must be soluble antigens which *a priori* might be expected to be distributed homogeneously through the cytoplasm Insoluble or membrane bound antigens, the heterogeneous distribution of which might be more readily understood, could only have been detected by the autoradiographic study.

Whether this unequal distribution of antigens in the *Drosophila* egg reflects the mosaic nature of the egg or is a trivial consequence of a more important gradient cannot be decided from these studies. The analysis of mutants showing disturbed embryonic development, for example temperature sensitive female sterile mutants, may help to resolve this problem.

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Induction of puffs in *Drosophila* salivary gland cells by mitochondrial factor(s)

IT is well established that specific puffs can be induced in the polytene chromosomes of *Drosophila* larvae by treatments such as heat shock[1–3] and by substances which interfere with respiration[4]; however, the intracellular signals produced in these conditions for the induction of specific puffs are not known. It has been suggested[5] that these signal(s) might come from mitochondria. I have investigated this possibility, using *Drosophila hydei.*

After heat shock a mitochondrial suspension was centrifuged and the supernatant injected into the cytoplasm of salivary gland cells. The responses of chromosomal regions 32A, 36A, 48BC and 81B, which are known to be sensitive to heat shock *in vivo* and *in vitro*[2], were analysed. Table 1 shows the results of analysis of regions 36A and 48BC. Injection of the 'injection medium', followed by 35 min incubation in Poels' medium[6] caused no significant changes in the relative diameters of these two regions. Microinjection into the salivary gland cells of the supernatant from mitochondria which had been subjected to 37 °C caused a significant increase in the relative diameter of regions 36A and 48BC. The extent of increase at region 36A was 11 %, comparable with that obtained from tissue which had been subjected to heat shock *in vitro.* The increase at region 48BC was 23 % over the normal relative diameter of 1.27, a value greater than that (13 %) from heat-shocked salivary glands Even larger puffs can be induced at this chromosome region by vitamin B_6 *in vivo*[7]. Injection of the supernatant from mitochondria which were kept at 20 °C failed to induce puffs at these regions. The puff-inducing ability of the mitochondrial supernatant was greatly reduced after boiling for 10 min. This suggests that the puff-inducing agent(s) require a certain structural configuration to be effective, or that they are complexes which can be dissociated by boiling. These puff-inducing agent(s) seem to have relatively high molecular weight as they could not be dialysed out. The possibility that these mitochondrial agent(s) were nonspecific adhering substances of cytoplasmic origin, being modified and released under heat shock, was also tested. Cytoplasm prepared by lightly homogenising tissue in the 'injection medium' followed by centrifuging at 12,000g to remove cell debris and mitochondria, was heated at 37 °C as described for mitochondria, and injected into intact gland cells At the two concentrations used (about 4 pg and 20 pg protein per cell), no changes were observed in the relative diameters of regions 36A and 48BC.

Figure 1 shows the dose response of the two chromosome regions to the amount of mitochondrial agent(s) injected. The maximum relative diameter of the puff induced at region 36A was attained after injection of about 1.25 pg protein; and at

Table 1 Relative diameter of chromosome regions 36A and 48BC

Experiment	No. of mitochondrial preparations tested	Chromosome region	Relative diameter	*t* Test
(1) Salivary gland cells injected with injection medium incubated in Poels' medium for 35 min at 20 °C	—	36A	1.00 ± 0 002*(46)	
		48BC	1.27 ± 0.03 (36)	
(2) Salivary gland in Poels' medium for 35 min, at 37 °C	—	36A	1 12 ± 0 01 (73)	
		48BC	1.44 ± 0.03 (74)	
(3) Salivary gland cell injected with supernatant from mitochondria kept at 20 °C for 35 min	8	36A	1 01 ± 0 01 (62)	Compared with (1), $P > 0.03$
		48BC	1.31 ± 0 03 (61)	$P > 0.02$
(4) Salivary gland cell injected with supernatant from mitochondria kept at 37 °C for 35 min	10	36A	1.11 ± 0.01 (84)	Compared with (1), $P < 0.005$
		48BC	1.56 ± 0.02 (68)	$P < 0.005$
(5) As in (4), but supernatant was heated at 100 °C for 10 min	3	36A	1.02 ± 0.01 (26)	Compared with (4), $P < 0.005$
		48BC	1.35 ± 0 03 (27)	$P < 0.005$
(6) As in (4), but supernatant was dialysed overnight	4	36A	1.10 ± 0.03 (53)	Compared with (4), $P > 0.2$
		48BC	1 53 ± 0.03 (49)	$P > 0 2$

Third-instar larvae of *D hydei* reared under standardised conditions[8] were used Mitochondria were prepared[9] from 300–400 mg mass isolated[10] salivary glands. The well washed (by repeated suspension and centrifugation) mitochondria, suspended in 0.8 ml of 'injection medium' (0.154 M KCl; 1.0 mM EDTA, *p*H 7 1), were divided into two equal portions. To simulate heat shock, one portion was kept at 37 °C for 35 min, the other portion at 20 °C. After heat shock the mitochondrial suspensions were centrifuged at 27,000g for 100 min at 4 °C. The supernatant is referred to as mitochondrial supernatant. Microinjection was carried out as before[11] The salivary glands, isolated in Poels' medium[6], were submerged in the same solution for microinjection Between eight and 15 cells at the distal end of each gland were injected each with $3–5 \times 10^{-5}$ µl of mitochondrial supernatant, the sister gland was used as control. For the purposes of comparison, the amount of material injected was expressed on a protein basis (2–4 pg per cell) Protein concentration was determined by absorption at 230 nm, thus the absolute amount of protein injected per cell is likely to be overestimated After injection the glands were incubated in Poels' medium[6] at 20 °C for 35 min, then fixed and stained with aceto-orcein; 6–8 injected cells were cut off and squashed The puffing activity of the chromosome regions was expressed as diameter relative to a reference chromosome band which remained inactive under various conditions. *Mean ± s e.m. Figures in parentheses indicate number of cells measured.

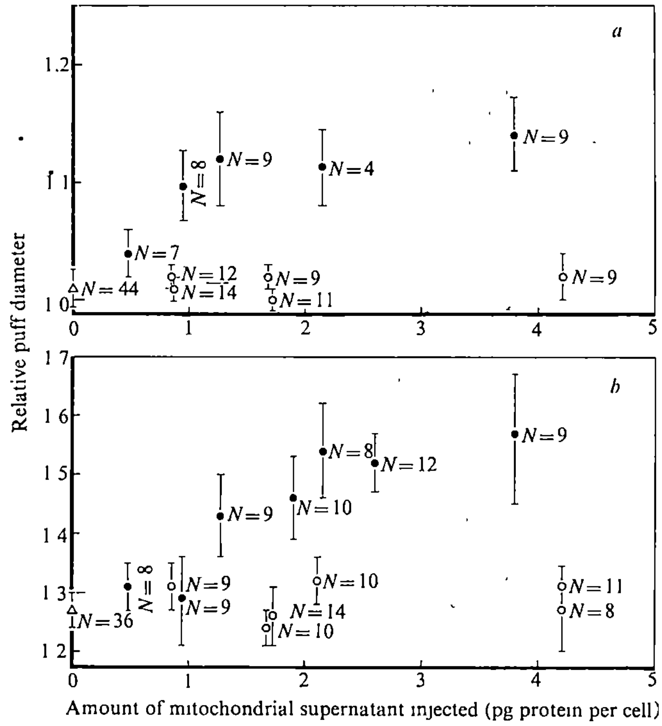

Fig. 1 Response of chromosome regions 36A (*a*) and 48BC (*b*) to injection of various amounts of mitochondrial supernatant from heat treated (●) and control (○) mitochondria. △, Cells injected with injection medium. Vertical bars represent s.e.m. *N*, Number of cells measured.

region 48BC at 2 pg protein per cell. With larger amounts no further substantial increases were observed.

Chromosome region 32A failed to respond to microinjection of the supernatant from the treated mitochondria. Region 81B showed only slight activity and so accurate comparison was not possible. The absence of puff formation at region 32A suggests that the simulation of heat shock of mitochondria *in vitro* does not have the same effect on the mitochondria as it would have in intact tissue, and that more than one puff-inducing agent is required for the induction of all four temperature puffs. The chemical nature of the mitochondrial agent(s) has not been established.

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Rapid loss of a novel phenylalanyl-tRNA on germination of *Neurospora crassa* conidia

SEVERAL theories attempt to explain the control of protein synthesis, and thus development at the level of transfer RNA[1,10]. We present evidence for changes in the isoaccepting species of phenylalanyl-tRNA during germination and growth of the fungus *Neurospora crassa*.

The tRNAs and aminoacyl-tRNA synthetases were isolated from conidia and mycelia as described in the legend of Fig. 1. The conidial tRNA preparation was esterified with ^{3}H-phenylalanine using the conidial synthetase, whereas ^{14}C-phenylalanine was used in the analogous mycelial system. The pattern shown in Fig. 1 was obtained on elution of the tRNAs from a benzoylated DEAE cellulose (BD-cellulose) column. The data were treated according to the method of Shearn and Horowitz[8], thus providing a direct comparison of the tRNA species even though they are labelled with different forms of radioactivity. Mycelial and conidial cultures were shown to contain two and three phenylalanyl-tRNAs, respectively. In addition to monitoring the radioactive amino acids, fluorescence and absorbancy values were also determined. Although the latter two measurements were performed in subsequent experiments, they are presented only in Fig. 1. Fluorescence measurements enable one to distinguish between the cytoplasmic and mitochondrial species of phenylalanyl-tRNA. The cytoplasmic species contain a fluorescent base while mitochondrial species do not (W. E. Barnett, personal communication). Those phenylalanyl-tRNAs which elute late in the salt gradient (fractions 90–100) do not fluoresce and are presumably of mitochondrial origin. The cytoplasm of conidia contains two species of phenylalanyl-tRNA (Phe-tRNA$_{I}^{c}$ and phe-tRNA$_{II}^{c}$), whereas the cytoplasm of mycelia contains only one (Phe-tRNA$_{I}^{m}$).

When the conidia are incubated in a medium which supports germination and growth, the minor species of phenylalanyl-tRNA (Phe-tRNA$_{II}^{c}$) disappears within 3 h. Although tRNAs are normally stable, their rapid loss is not unknown[9].

The levels of methyl-group acceptance are reported[11] to vary in conidial and mycelial populations of tRNA. To test the possibility that the loss of Phe-tRNA$_{II}^{c}$ may result from its conversion by methylation to Phe-tRNA$_{I}^{c}$, the following experiments were performed. Preparations of either conidial or mycelial tRNAs were first esterified with ^{3}H-phenylalanine, then subjected, *in vitro*, to methylation using ^{14}C-S-adenosylmethionine as the methyl donor in the presence of the nuclease competitive inhibitor 2′,3′-cytidine monophosphate (2′,3′-CMP). Control experiments involved incubation of the esterified tRNAs in the methylation reaction mixture minus the methyl donor and 2′,3′-CMP.

Figure 2*a* shows the pattern of methyl-group acceptance in a population of conidial tRNAs. Because the Phe-tRNA$_{II}^{c}$ does not disappear, conversion by methylation is ruled out as a mechanism. Since the levels of the two Phe-tRNA species resolved by the BD-cellulose chromatography were quantified by amino acid charging saturation experiments as well as by fluorescence measurements, the differences in methyl group acceptance between the two forms indicates that Phe-tRNA$_{II}^{c}$ is undermethylated with respect to Phe-tRNA$_{I}^{c}$. Methylation of tRNAs other than Phe-tRNA results in an accumulation of these ^{14}C-labelled compounds in the 0.9 M NaCl wash. When the conidial tRNAs were incubated in the methylase reaction mixture minus the methyl donor and 2′3′-CMP, a measure of endogenous nuclease activity was obtained. As a result of the incubation, Phe-tRNA$_{I}^{c}$ remained at its initial level but the Phe-tRNA$_{II}^{c}$ level was decreased (Fig. 2*b*). Furthermore, some of the ^{3}H-phenylalanine eluted very early in the salt gradient and the remainder was removed in the 20% ethanol wash. The products of the nuclease attack were found in the 20% ethanol wash, whereas the radioactivity which appeared early in the salt wash was free phenylalanine resulting from deacylation of the charged Phe-tRNAs due to the high *p*H of the reaction mixture. Figure 2*c* and *d* is analogous to the experiments described above except that the mycelial tRNA preparation was used. The important features of these latter two experiments are: (1) only a small amount of ^{14}C-methyl label is accepted by the mycelial tRNA^{Phe} suggesting this species is fully methylated *in vivo*; (2) the level of mycelial tRNA^{Phe} is not altered by incubation in the methylase reaction mixture minus the methyl donor and

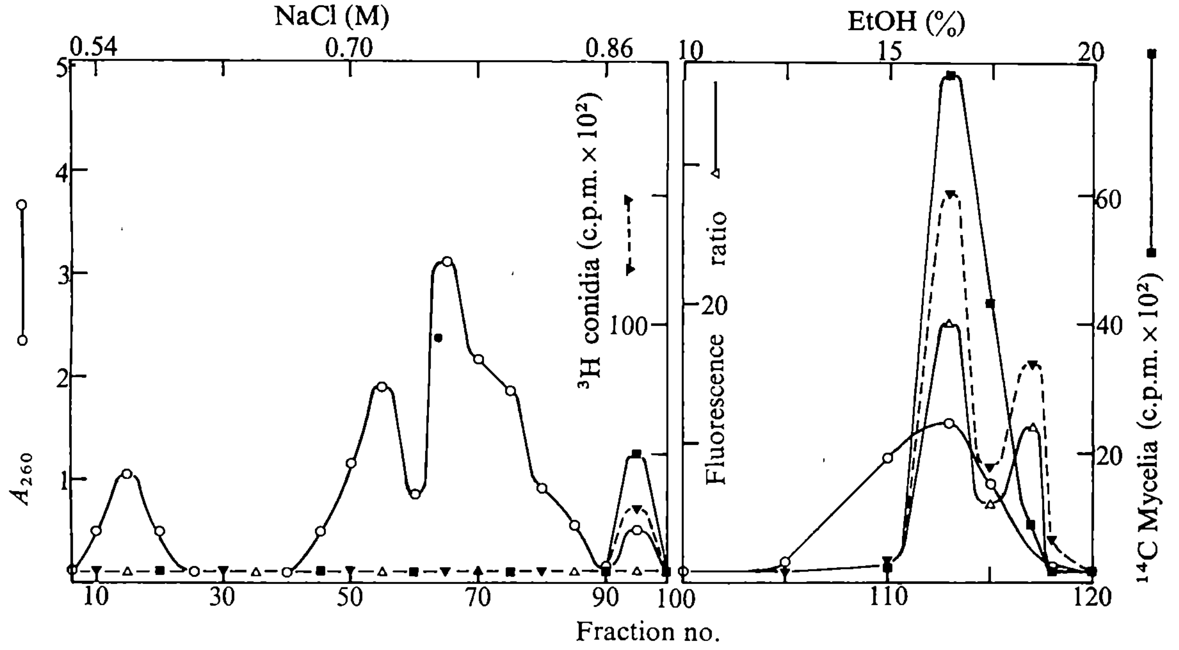

Fig. 1 BD-cellulose column chromatography of phenylalanyl-tRNAs from *N. crassa* conidia and mycelia. Conidia and mycelia, from the wild type strain 74-OR-23-1A, were used as sources of tRNA and aminoacyl-tRNA synthetase. The tRNA was extracted by a modified method of Holley *et al.*[3]. The reaction mixture contained 20 ml of 0.1 M Tris buffer, *p*H 7.4, containing 0.001 M $MgCl_2$ and 0.005 M KCl and 15 ml of water-saturated phenol per 10 g wet weight of cells. The aminoacyl tRNA synthetases were prepared using the method of Haskinson and Khorana[4]. The reaction mixture for the assay of amino acid acceptance was patterned after the methods of Reeves *et al.*[6] and of Shearn and Horowitz[7]. The final concentrations of reactants in the 2.5-ml assay were: Tris-HCl, *p*H 7.5, 50 mM; $MgCl_2$, 15 mM; NaEDTA, 1 mM; Na ATP, 5 mM; ^{14}C or ^{3}H amino acid, 30 µM (specific activity was 50 µCi µmol^{-1} for ^{14}C and 250 µCi µmol^{-1} for ^{3}H); 40–60 A_{260} units of tRNA per ml; and synthetase preparation (2.0 mg ml^{-1}). BD-cellulose column chromatography was carried out as described by Gilliam *et al.*[2] The column was equilibrated with a buffer containing 0.6 M NaCl, 0.01 M $MgSO_4$ and 0.01 M NaAc, *p*H 4.5. The tRNAs were eluted with a 400-ml linear gradient of 0.6 M to 0.9 M NaCl in the above buffer. After completion of the salt gradient, a 200-ml, 10—20% ethanol gradient in the 0.9 M NaCl buffer was applied. Fractions of 4 ml were collected and assayed for radioactivity, absorbance and fluorescence. The fluorescence ratio of 1 ml in 0.1 M K_3PO_4 was measured in a Beckman ratio fluorometer, model 772. The excitation wavelength was 310 nm and fluorescence was measured in the 410–460-nm range.

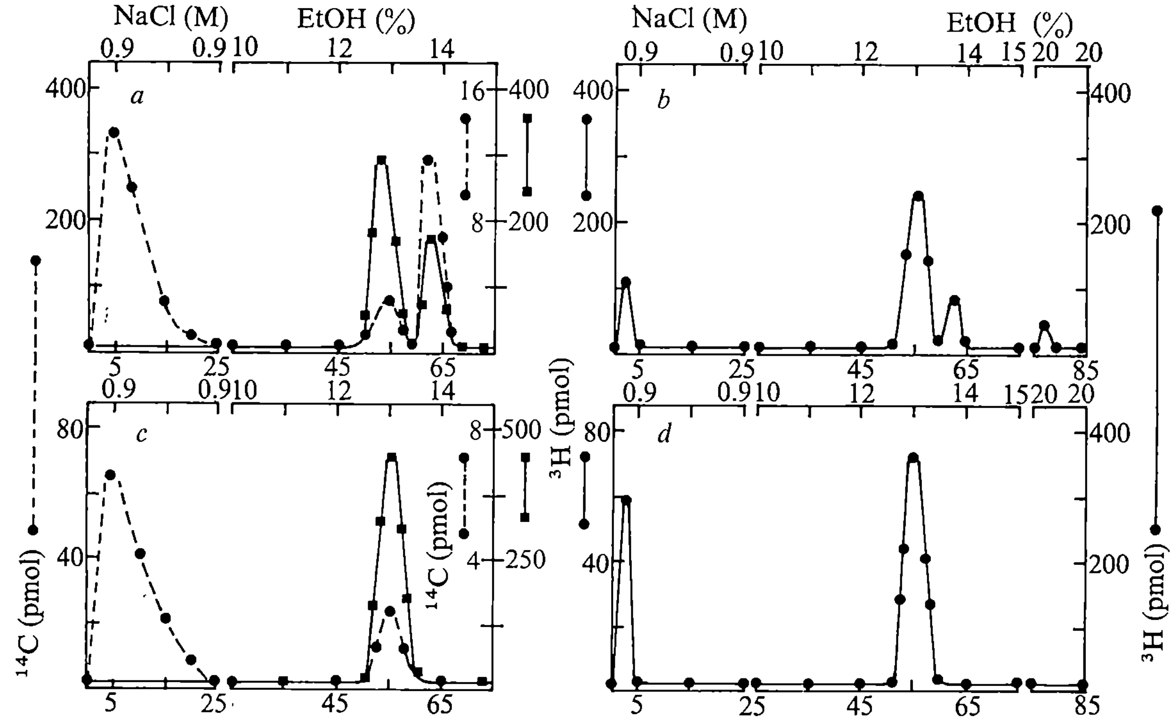

Fig. 2 Effect of methylation on phenylalanyl-tRNA isolated from *N. crassa* conidia and mycelia. The assay for methyl group acceptance by tRNA was patterned after experiments described by Wong *et al.*[11]. The tRNA was esterified and prepared for BD-cellulose chromatography as described in the legend of Fig. 1; however, before application to the column, the tRNA was added to the following reaction mixture: Tris-HCl, *p*H 7.0, 20 µM; mercaptoethanol, 2 µM; $MgCl_2$, 2 µM; tRNA, 100 µg; enzyme, 0.5 mg; ^{14}C-methyl S-adenosylmethionine, 2 µM (specific activity 50 mCi mmol^{-1}). The tRNA preparation was applied to the BD-cellulose column, washed with 0.9 M NaCl buffer, and then eluted with a 10–15% ethanol gradient in 0.9 M NaCl buffer. *a*, Conidial tRNA esterified with ^{3}H-phenylalanine (——); and methylated with ^{14}C (– – – –) in the presence of 1 mM 2′,3′-CMP. *b*, Conidial tRNA esterified with ^{3}H-phenylalanine (——) and incubated in a methylation reaction mixture minus the methyl donor and minus CMP. *c*, Mycelial tRNA esterified with ^{3}H-phenylalanine (——); and methylated with ^{14}C (– – – –), in the presence of 1 mM 2′,3′-CMP. *d*, Mycelial tRNA esterified with ^{3}H-phenylalanine (——); and incubated in methylation reaction mixture minus the methyl donor and minus CMP.

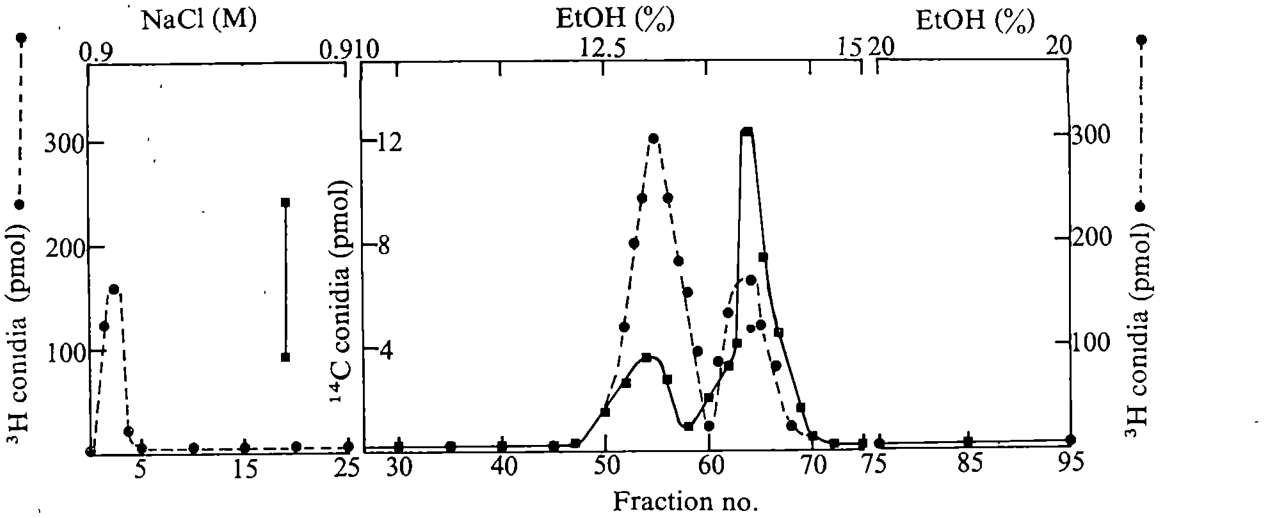

Fig. 3 Protection by methylation of phenylalanyl-tRNA of *N. crassa* from nuclease attack Conidial tRNA^{Phe} esterified with ^{3}H-phenylalanine and then methylated using a ^{14}C-methyl donor (fractions 45–70 from Fig. 2*a*) were reincubated in the methylation reaction mixture but minus the 2′,3′-CMP, then rechromatographed on the BD-cellulose column. – – – –, Phenylalanine-^{3}H, ——, methyl group-^{14}C.

CMP. Since no nuclease products are evident, the free phenylalanine which appears early in the salt wash is due to deacylation rather than nuclease attack of the esterified tRNA^{Phe}.

Although methylation did not convert Phe-tRNA$_{II}{}^c$ to Phe-tRNA$_I{}^c$, *in vitro*, the data from these four experiments indicate that methylation may protect under-methylated species of tRNA from nuclease attack. Whether methylation or 2′,3′-CMP protected Phe-tRNA$_{II}{}^c$ cannot be ascertained from the above experiments. To distinguish between the two possibilities, the following experiment was performed. The tRNAs in fractions 45–70 from Fig. 2*a* were collected, precipitated, and resuspended in the methylation reaction mixture but minus the 2′,3′-CMP. The fractions were rechromatographed and the elution pattern is shown in Fig. 3. The ^{3}H-phenylalanine appeared only as the free amino acid in the early portion of the salt wash and was not associated with nuclease products in the 20% ethanol wash. These data indicate that under-methylated Phe-tRNA$_{II}{}^c$ was protected from nuclease attack by *in vitro* methylation. Thus, the rapid disappearance of Phe-tRNA$_{II}{}^c$ observed *in vivo* may be the result of nuclease action on this labile species of Phe-tRNA.

In an earlier report[5], we showed that phenylalanine exists as a unique NH_2-terminal amino acid in conidia. The transient Phe-tRNA of conidia might function in conidia-specific synthesis of these proteins. If so, a mechanism for its inactivation or destruction on conidial germination would seem to be an essential feature of regulation of this specific protein synthesis. The synthesis or maintenance of levels of glycoprotein associated with amino acid transport in conidia might be related to the transient Phe-tRNA. This glycoprotein is known to have phenylalanine as an NH_2-terminal amino acid (W. D. Stuart, personal communication) and amino acid transport mediated by this permease is known to decrease after germination of conidia. In conclusion, this transient tRNA offers a convenient handle for studying the operation of regulatory mechanisms involved in the transition from the conidial to the vegetative state of *Neurospora*.

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Deletion of the β-globin structure gene in hereditary persistence of foetal haemoglobin

Hereditary persistence of foetal haemoglobin (HPFH) is a condition characterised by the persistence of a high percentage of foetal haemoglobin into adulthood[1]. The homozygous state has been described in the Negro[2–4] and the Indian[5]; only haemoglobin F is present, and haemoglobins A and A_2 are absent. It has been suggested that in this syndrome γ-chain synthesis fails to switch to β-chain synthesis after birth. The reason for this failure is unknown, although deletions of the β- and δ-structural genes have been proposed[6]. We report here analysis of the DNA from a patient with the homozygous form of HPFH, using radioactive DNAs complementary to β- and γ-globin mRNA (β and γcDNAs); the results are compatible with deletion of the β-globin structural locus.

The annealing of β- and γcDNA to DNA is shown in Fig. 1. The βcDNA annealed to 65% with normal DNA, but only to 35% with HPFH DNA. In contrast, γcDNA annealed equally to normal and HPFH DNA (90%).

In man, from the mode of inheritance of β-globin structural abnormalities, there is believed to be a single β structural locus[8], while structural variation in the γ-globin chains demonstrates that there are two to four γ structural loci[9]. Analysis by DNA hybridisation has confirmed these gene numbers[10]. The smaller extent of hybridisation to the normal DNA of βcDNA compared with γcDNA at the ratio cDNA:DNA used in these experiments is consistent with the larger number of γ-globin genes. The normal rate of hybridisation of γcDNA to both DNAs suggests that the γ-globin genes in HPFH are intact. This agrees with the finding that the foetal haemoglobin of this patient contains both the $^G\gamma$ and the $^A\gamma$ types[9]. The significantly decreased

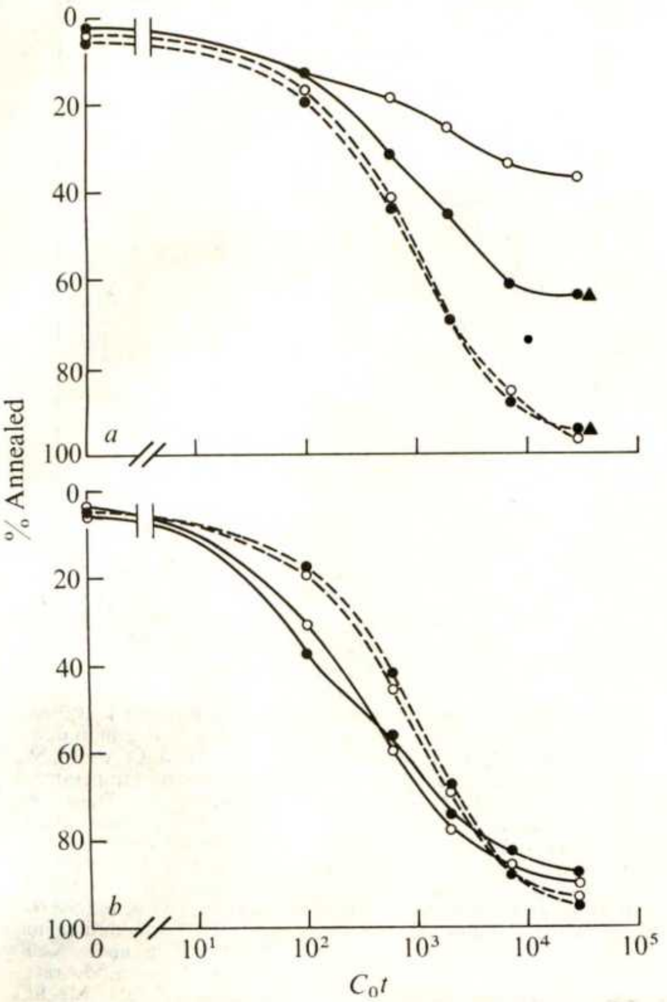

Fig. 1 Rate of annealing of normal and HPFH DNA. DNA was prepared from fibroblasts cultured from a skin biopsy of an HPFH patient, and from the skin fibroblasts and white blood cells (WBC) of two non-thalassaemic controls, as before[7]. βcDNA was transcribed from the 10S RNA (α : β = 1 : 8 by translation) from the reticulocytes of a patient with haemoglobin H disease, and γcDNA was transcribed from the reticulocyte RNA (α : β : γ = 0 : 1 : 8) of a patient with hydrops fetalis. ^{32}P-α-dCTP was used as the label, and the methods for synthesis and isolation of the cDNA were as previously described[7]. Both cDNAs were used without further enrichment of the β or γ sequences. Each 10-μl reaction mixture contained 400 c.p.m. (3.7 pg) of ^{32}P-labelled β or γcDNA, 75 μg of cellular DNA, 500 c.p.m. of ^{3}H-dCTP labelled HeLa unique sequence DNA as internal controls, 0.5 M NaCl, 0.04 M Tris-HCl (*p*H 7.4) and 1.5 mM EDTA, and was incubated for 0–72 h at 68 °C. Hybrids were assayed with hydroxylapatite[7]. *a*, βcDNA; *b*, γcDNA. ●, Normal WBC DNA; ○, HPFH fibroblast DNA; —, cDNA; - - -, unique sequence Hela DNA; ▲, shows that the annealing of βcDNA to normal skin fibroblast DNAs was identical to the WBC DNA.

hybridisation of βcDNA in HPFH is compatible with the deletion of all or part of the single β locus. There are several possible reasons why some βcDNA annealed to the HPFH DNA. First, 10–15% can be attributed to the α sequence known to be present in the βcDNA, Second, some portion of the β-structural gene may remain undeleted. Third, nonspecific αcDNA hybrids with low thermal stability have been found in hydrops fetalis where the α-globin structural genes are deleted (ref. 7 and unpublished work of Y.W.K.). Similar nonspecific hybridisation of the βcDNA may occur in the absence of β-globin structural genes.

We cannot infer from these data whether or not the δ-globin gene is affected, as the extent of cross hybridisation between β and δ sequences is not known. The absence of haemoglobin A_2 in homozygous HPFH suggests involvement of the δ-globin gene also, but this may not necessarily be due to deletion, as other mechanisms resulting in the absence of δ-chain synthesis may be possible.

In conclusion, the decreased hybridisation of the βcDNA to HPFH DNA indicates that all or a large portion of the β-globin locus is deleted in this syndrome.

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Effect of DTNB light chain on the interaction of vertebrate skeletal myosin with actin

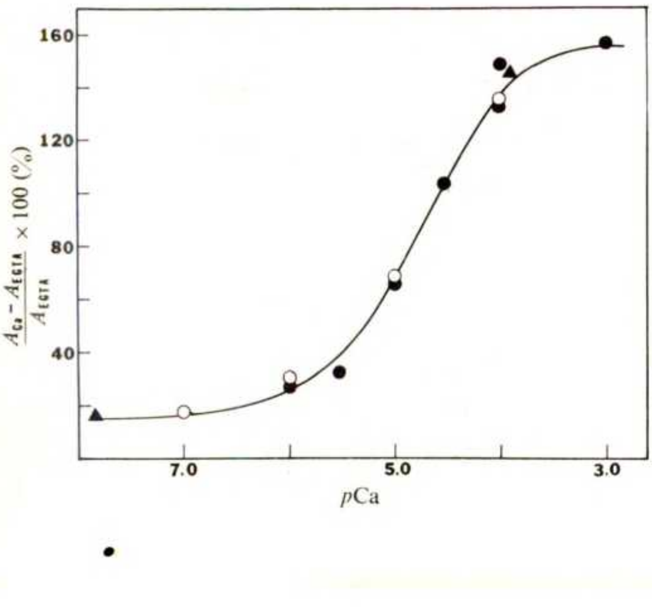
MYOSIN from vertebrate skeletal muscles contains four low molecular weight subunits which are thought to play a functional role in the enzymic activity of myosin[1,2] Removal of 'alkali light chains' (21,000 and 17,000 g mol^{-1}) results in complete loss of ATPase activity, but dissociation of 'DTNB light chain' (18,000 g mol^{-1}) leaves the enzymic activity of myosin essentially unaffected[3–6]. Since harsh solvents are required to dissociate alkali light chains from heavy chains (as the name implies), it is unclear whether the activity loss is related primarily to denaturation of the heavy chain or to removal of the light chain[7].

The only light chain for which a function has been clearly defined is that associated with myosin from molluscan muscles. It is now well documented that calcium regulation in molluscs is through the thick filament; the thin filaments lack troponin and do not bind calcium[8]. Thus the 'EDTA light chain' of scallop myosin is a true regulatory subunit in that it controls whether the muscle is in an active or a resting state[9]. No comparable calcium sensitivity has been shown for vertebrate skeletal myosin, although rabbit myosin[10], as well as isolated DTNB light chains[11], can bind calcium in the 10^{-5} M range. Nevertheless, some effects of calcium on the properties of myosin filaments have been observed. The sedimentation coefficient of isolated, native thick filaments increases by about 3% with increasing calcium levels[11]. X-ray diffraction patterns from contracting muscle indicate a movement of cross bridges on activation, which is independent of the presence of actin[12]. Both studies suggest a conformational change occurring within the thick filament. The biological significance of these observations however, is unclear; and an involvement of the DTNB light chain in these structural changes has not been unequivocally demonstrated. We present here data showing that the DTNB light chain is directly involved in the interaction between myosin and actin, and moreover, that the strength of this interaction depends on the concentration of calcium present in the medium.

At some stage in the contractile cycle, myosin is probably bound to actin in a 'rigor'-type linkage. The energy needed to break this bond is derived from the binding of ATP to myosin. A value for the association of myosin and actin in the absence of ATP has been estimated to be no less than 10^6 M^{-1} and may be considerably higher[13,14]. We have redetermined this parameter by analysing the binding of heavy meromyosin (HMM) and heavy meromyosin subfragment 1 (S1) to F actin in the analytical ultracentrifuge, using the scanning absorption optical system at 230 nm. By adding an increasing concentration of enzyme to a fixed amount of F actin and sedimenting the complex, the free concentration of enzyme can be measured in the supernatant and a binding isotherm constructed. The details of these experiments will be presented elsewhere, but the principal conclusions may be summarised as follows: the association constant for HMM and actin is 1×10^6–4×10^6 M^{-1} in 0.1 M KCl, 10 mM imidazole, *p*H 7.0, 1 mM $MgCl_2$, 5 mM Pi; the corresponding value for S1 is about 10^5 M^{-1}. Unexpectedly, we found that these values increased nearly tenfold on addition of 0.3 mM EGTA to the basic solvent, whereas 0.1 mM Ca^{2+} caused a significant reduction in binding[15]. Figure 1 shows that the midpoint of this transition in binding occurs at about *p*Ca 5. Although this amount of calcium is an order of magnitude higher than that required to activate skeletal muscles, it is still well within the physiological range of calcium reached in a contracting muscle cell.

Having shown a calcium response in vertebrate skeletal myosin, the question arises as to the site of calcium action. Since calcium affects the interaction with actin, an involvement of the head region of myosin is indicated; and, indeed, both HMM and S1 are sensitive to calcium. The magnitude of the response, however, varied from one preparation to the next, suggesting possible proteolytic degradation of essential sites. This variation was most pronounced for S1, where some preparations gave less than 20% of the effect observed with myosin.

Fig. 1 Effect of calcium concentration on the extent of actomyosin formation. Rabbit myosin (1.27 mg ml^{-1}) was mixed with F actin (0.24 mg ml^{-1}) in 0.34 M KCl, 10 mM imidazole (*p*H 7.5), 5 mM KPi and 1 mM $MgCl_2$ in a volume of 2 ml. The calcium level was adjusted by Ca-EGTA–EGTA buffers. The reaction mixture was stirred for 30 min in the cold, before centrifugation at 40,000 r.p.m. in the analytical ultracentrifuge. The concentration of unbound myosin in the supernatant was determined at 280 nm with the photoelectric scanner (Model E). Each point represents the difference between the absorbance (A_{Ca}) in the presence of calcium and in its absence (A_{EGTA}), relative to the absorbance in 0.3 mM EGTA. *p*Ca is the negative logarithm of the molar concentration of free calcium. Five measurements could be made simultaneously using the AN-G rotor with the multiplexer accessory. ○, ●, ▲, Different preparations.



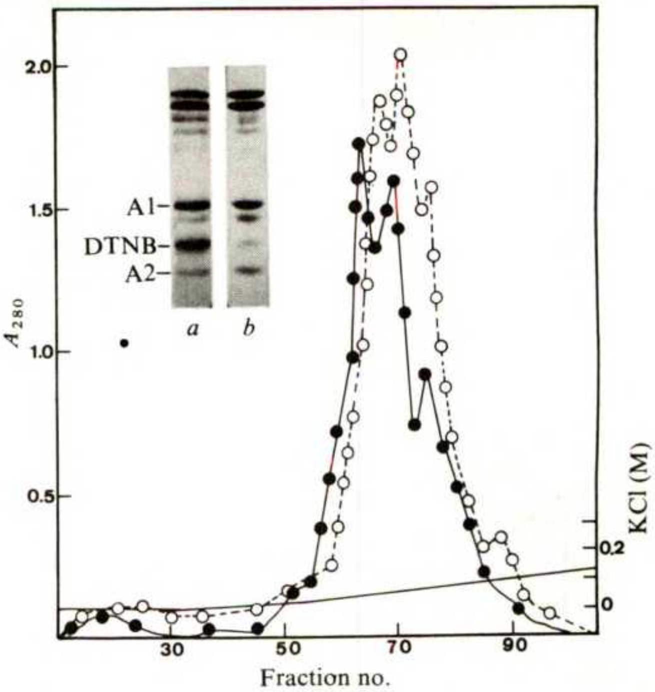


Fig. 2 Chromatographic purification of subfragment 1. Rabbit myosin at about 2% was dialysed against 0.2 M ammonium acetate, *p*H 7.2, in the presence of either 2 mM $MgCl_2$ or 2 mM EDTA. The precipitated myosin was digested at room temperature for 7 min with 0.03 mg ml^{-1} papain (Worthington). Digestion was stopped by adding iodoacetic acid to a final concentration of 1.0 mM. Insoluble material was removed by centrifugation and the supernatant dialysed against 0.05 M Tris-HCl (*p*H 7.9), 0.5 mM dithiothreitol, containing either 2 mM $MgCl_2$ or 2 mM EDTA. About 450 mg subfragment 1 was applied to a 2.5×60 cm DEAE-cellulose column (Whatman DE52) equilibrated in the same buffer. The protein was eluted with a linear KCl gradient (0.0–0.5 M KCl in a total volume of 2 l) at a flow rate of 45 ml h^{-1}, collecting 6 ml per tube. Fractions 60–70 of Mg–S1 (●) and 60–72 of EDTA–S1 (○) were pooled and the material salting out between 49 and 58% saturated ammonium sulphate retained for further experiments. Representative samples of Mg–S1 (*a*) and EDTA–S1 (*b*) at 15-μg loads on 10% SDS–polyacrylamide gels are shown above.

The standard procedure for preparing S1, the 'single head' of myosin, is to digest a suspension of myosin with a low concentration of papain[14]. S1 prepared in this manner shows one symmetrical peak in the ultracentrifuge, has a high level of ATPase activity, and is regulated by the troponin–tropomyosin system. Gel electrophoresis of rabbit S1 gives two bands of about 90,000 and three light chain components plus an extra band (which probably originates from cleavage of the heavy chain)[16] in the 20,000 g mol^{-1} region. Densitometry of such gel patterns shows that less DTNB light chain is present in S1 than occurs per head of native myosin, a finding consistent with radiochemical studies indicating a loss of ~0.5 mol DTNB light chain per mol S1 (ref. 3).

The susceptibility of DTNB light chain to proteolysis is reminiscent of the early difficulties in preparing S1 from scallop myosin[9]. Depending on whether divalent cations were present or absent in the digestion medium, EDTA light chain remained intact or was completely degraded by papain. These results suggested that perhaps the stability of DTNB light chain might be similarly affected by the presence of magnesium ions. Consequently the procedure for digesting rabbit myosin to S1 was modified to include 2 mM $MgCl_2$ or 2 mM EDTA in all solutions (see legend to

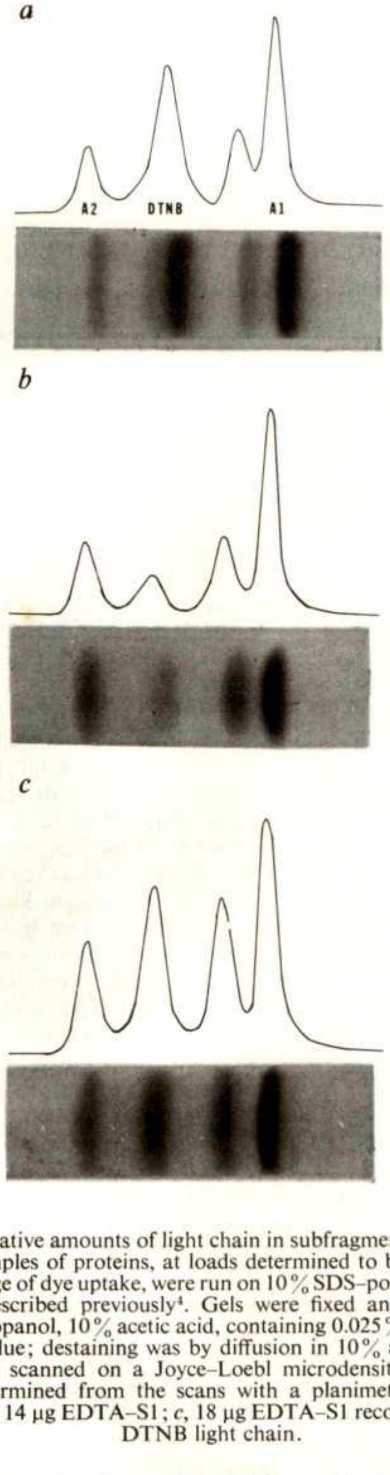


Fig. 3 Relative amounts of light chain in subfragment 1 preparations. Samples of proteins, at loads determined to be within the linear range of dye uptake, were run on 10% SDS–polyacrylamide gels as described previously[4]. Gels were fixed and stained in 25% 2-propanol, 10% acetic acid, containing 0.025% Coomassie brilliant blue; destaining was by diffusion in 10% acetic acid[18]. Gels were scanned on a Joyce–Loebl microdensitometer, and areas determined from the scans with a planimeter. *a*, 14 µg Mg–S1; *b*, 14 µg EDTA–S1; *c*, 18 µg EDTA–S1 recombined with DTNB light chain.

Fig. 2 for details). Chromatographic profiles of Mg-S1 and EDTA-S1 are illustrated in Fig. 2, together with electrophoretic patterns of the pooled fractions. These gels provide clear evidence that S1 prepared in the presence of magnesium ions has a significantly higher proportion of DTNB light chain than S1 digested in EDTA. In fact, densitometry of the chromatographed samples showed that Mg-S1 has approximately the same content of DTNB light chain as myosin (Fig. 3 and Table 1). The relative amount of alkali 1 to alkali 2 was fairly constant in both types of subfragment, and gave a ratio similar to that found for native rabbit myosin[17].

A preliminary characterisation of Mg-S1 and EDTA-S1 revealed few major differences in their properties: Both appeared monodisperse in the ultracentrifuge and sedimented at about equal rates, although inspection of the shape of the sedimentation velocity profile suggested a possible difference in frictional coefficients. The Ca^{2+}- and EDTA-activated ATPases for the two proteins were in the range of 3 and 10 s^{-1}, respectively; and the actin-activated ATPase was between 13 and 16 s^{-1}. The activity of both preparations was inhibited by troponin–tropomyosin to the extent of 70–80% in the absence of calcium. These results confirm previous reports that DTNB light chain is not involved in actin-linked regulation[18].

The most significant difference between the preparations of Mg-S1 and EDTA-S1 was in their response to calcium,

Table 1 Relative amounts of light chain in subfragment 1

Subfragment	Experiment	Alkali 1	DTNB light chain	Alkali 2
EDTA-S1	1	1.15	0.29	0.85
	2	1.29	0.36	0.71
	3	1.14	0.48	0.86
Mg-S1	1	1.28	1.79	0.72
	2	1.21	1.71	0.79
	3	1.71	2.00	0.29
Recombined	1	1.23	0.66	0.77
	2	1.20	1.12	0.80
	3	1.03	0.81	0.97

Amount of each light chain relative to the total light chain content was determined by dividing the densitometer area by the molecular weight and normalising values to mol (A1+A2 = 2). Each value represents an average of 4–6 determinations. Conditions for electrophoresis were as in Fig. 3. "Recombined" subfragment was prepared by adding total light chains (300 mg) or purified DTNB light chain[18] (80 mg) to EDTA-S1 (300 mg), and separating the subfragment from the uncombined light chains by ion-exchange chromatography as in Fig. 2.

Fig. 4 Ultracentrifuge scanner traces of the binding of subfragment 1 to F actin as a function of calcium. Mg–S1 (0.33 mg ml^{-1}) or EDTA–S1 (0.33 mg ml^{-1}) in 0.10 M KCl, 10 mM imidazole (*p*H 7.0), 5 mM KPi, 1 mM $MgCl_2$, containing either 0.1 mM $CaCl_2$ or 0.3 mM EGTA, was added to F actin (0.24 mg ml^{-1}) in a final volume of 2 ml. The mixture was stirred for 30 min before centrifugation at 40,000 r.p.m. The concentration of free enzyme remaining in the supernatant was determined at 230 nm from the magnitude of the plateau region, and corrected for unpolymerised actin. *a* and *b*, Mg–S1 in the presence and absence of calcium, respectively. *c*, EDTA–S1; scan was the same for calcium or EGTA.

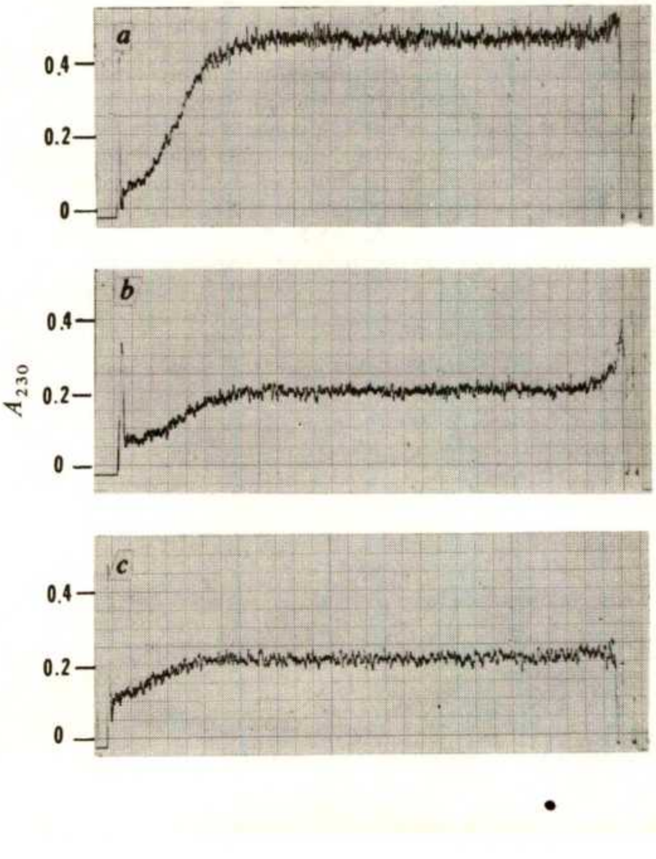


Table 2 Effect of calcium on binding of subfragment 1 to actin

Subfragment	Experiment	A_{230} 0.1 mM $CaCl_2$	 0.3 mM EGTA	Calcium sensitivity (%)
EDTA–S1	1	0.28	0.28	—
	2	0.37	0.36	—
	3	0.24	0.24	—
Mg–S1	1	0.76	0.31	147
	2	0.78	0.32	148
	3	0.46	0.20	130
Recombined	1	0.56	0.28	100
	2	0.58	0.31	87
	3	0.45	0.32	41

Absorbances for subfragments were obtained as in Fig. 4. "Calcium sensitivity" is expressed as $((A_{Ca}-A_{EGTA})/A_{EGTA})\times 100$.

using the binding of S1 to actin as an assay. Figure 4 shows that when Mg-S1 was reacted with actin in the presence of calcium, twice as much enzyme remained unbound in the supernatant as when a chelating agent was present. In contrast, the amount of free EDTA-S1 remained invariant with calcium concentration. Several such experiments are summarised in Table 2. Although the difference in DTNB light chain content is the most striking feature of the S1 preparations, it must be recognised that minor modifications in the heavy chain could be responsible for the unresponsiveness of EDTA-S1 to calcium. It was therefore important to demonstrate that addition of DTNB light chain to EDTA-S1 would restore the calcium effect. Preliminary efforts at recombination are shown in Tables 1 and 2 and Fig. 3. In spite of difficulties in achieving full recombination of DTNB light chain, the results do indicate a partial restoration of the calcium response.

The experiments reported here demonstrate that the DTNB light chain is directly involved in the binding of calcium to vertebrate skeletal myosin. These findings should not be interpreted to mean that a second control mechanism necessarily exists in vertebrate muscles. The effect of calcium observed here is to weaken rather than to strengthen the actomyosin interaction. Furthermore, the level of calcium required for this effect is considerably higher than that needed to activate muscle. Nevertheless, the results show that the properties of the myosin head can be affected by the DTNB light chain, and perhaps it is not too unrealistic to speculate that subunit interactions of this kind may have an important role in the attachment of a cross bridge to actin during the contractile cycle.

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Fluorescence lifetimes in the photosynthetic unit

THE fluorescence intensity of a photosynthetic organism at any instant is a measure of the concentration of excited chlorophyll and it has, therefore, been one of the most studied of all the phenomena related to photosynthesis. Fluorescence lifetimes are a direct measure of the rate of energy transfer from the light collecting unit to the chemical trap. Lifetimes in the nanosecond region have been measured by many workers, following the pioneering work of Brody and Rabinowitch[1], but these have been near the limit of time resolution of the apparatus used. Much shorter average lifetimes have been measured by phase shift methods[2]. The picosecond pulses which are available from mode-locked lasers have now made possible a fully time-resolved study of fluorescence decay in the photosynthetic units of algal chloroplasts and their fragments.

The first laser pulse measurements on picosecond fluorescence lifetimes used the electro-optical shutter[3–5] but the irreproducibility of pulse intensity makes this a rather imprecise method. The streak camera[6,7] has greatly improved the precision but much of this is lost if photographic recording is used. With silicon-target, vidicon multichannel detection, which we have used in this work, the streak camera provides the best precision available at present for picosecond fluorescent kinetic studies. The picosecond pulses were generated from a mode-locked Nd^{3+} glass oscillator and the second harmonic at 530 nm was produced with 10–15% efficiency by a temperature-tuned caesium dihydrogen arsenate crystal. The train of pulses (approximately 100 with pulse separation of 6.7 ns) was directed at 45° on to the front surface of a cuvette (1 mm thick) containing the sample. The fluorescence passed through a Barr and Stroud DB5 CYAN filter to the slit of an Imacon streak camera which had an S20 photocathode. The fluorescence from one pulse near the centre of the train was selected for examination; this pulse had a duration 5–10 ps and its energy was typically 200 μJ. The signal was recorded on a 500-channel optical multichannel analyser and was calibrated both in intensity and in time.

Fluorescence lifetimes of algae, chloroplasts and chloroplast fragments have been measured. The algae used were *Chlorella pyrenoidosa* and *Porphyridium cruentum* grown in liquid culture[8] and collected during the logarithmic growth phase. Chloroplasts were isolated from spinach leaves (*Spinacea oleracea*) by a method previously reported[9] except they were subjected to osmotic shock before being suspended in the experimental medium which consisted of 0.33 M Sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA and 50 mM HEPES brought to *p*H 7.6 with KOH. Subchloroplast particles were prepared by the method of Anderson and Boardman[10]. Measurements with algae were made with 10–15% suspensions, and with chloroplasts and subchloroplast particles the chlorophyll, as determined by Arnon's method[11], was about 3 mg ml^{-1}. Fluorescence emission and yields were also measured in the nanosecond region of the decay using a conventional single-photon counting system.

Figure 1 shows the fluorescence decay curves for the two algae and Fig. 2 shows the decays for untreated spinach chloroplasts and for the same chloroplasts treated with 20 mM sodium dithionite, which reduces the activity of the chemical traps. The decays of *Chlorella*, *Porphyridium* and untreated chloroplasts were exponential from 20 to 300 ps after excitation and their decay times are given in Table 1. Beyond 300 ps there

Table 1 Fluorescence lifetimes of algae and chloroplasts

	τ (ps)
Chlorella	108 ± 10
Porphyridium	92 ± 10
Chloroplasts	134 ± 10
Chloroplasts + dithionite	130 ± 20 $1,450 \pm 150$

is evidence of a very weak longer lived component of the fluorescence.

Dithionite treatment of chloroplasts had little effect on the main component of fluorescence but markedly increased the intensity and lifetime of the longer lived component and the total yield of fluorescence. The longer lived component was measured on our nanosecond single-photon counting apparatus (1.6 ns) as well as on the picosecond apparatus (1.45 ns) and the decay was exponential. These lifetimes agree with previous measurements in this region[12].

Sub-chloroplast particles centrifuged at 1,000, 10,000, 50,000 and 144,000*g* gave increasing intensity of the shorter lived component over that of the longer lived one. This is probably to be attributed to the higher proportion of photosystem I (PS I) in the high *g* centrifuged particles. This, together with the effect of dithionite on the longer lived component of the chloroplast, suggests that the shorter and longer decays are to be identified with PS I and PS II respectively.

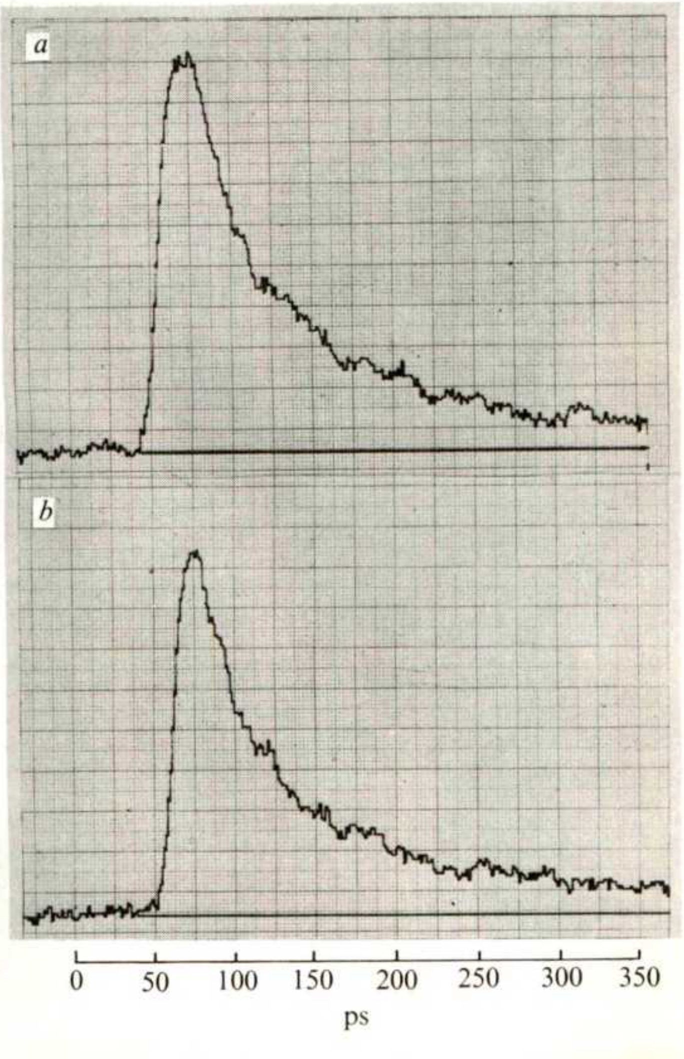
In the chloroplasts treated with dithionite, the two fluorescence decays (attributed to PS I and PS II) are quite distinct and it has been possible to make quantitative measurements on their relative contributions to the fluorescence. The relative numbers, *f*, of chlorophyll *a* molecules initially excited in the two systems is given directly by the ratio of initial fluorescence intensities of the two components, which is 4.5, the shortest component being the more intense. From this, and the measured lifetimes, the fluorescence yield of each system may be calculated from the relationships:

$$\varphi_{\rm I} = f_{\rm I}(\tau_{\rm I}/\tau_0)$$

$$\varphi_{\rm II} = f_{\rm II}(\tau_{\rm II}/\tau_0)$$

where $\varphi_{\rm I}$, $\tau_{\rm I}$ and $f_{\rm I}$ are the fluorescence yield, the lifetime and the fraction of excitation transmitted to chlorophyll *a* molecules of

Fig. 1 Fluorescence decay of intact algae. *a*, *Porphyridium cruentum*; *b*, *Chlorella pyrenoidosa*.



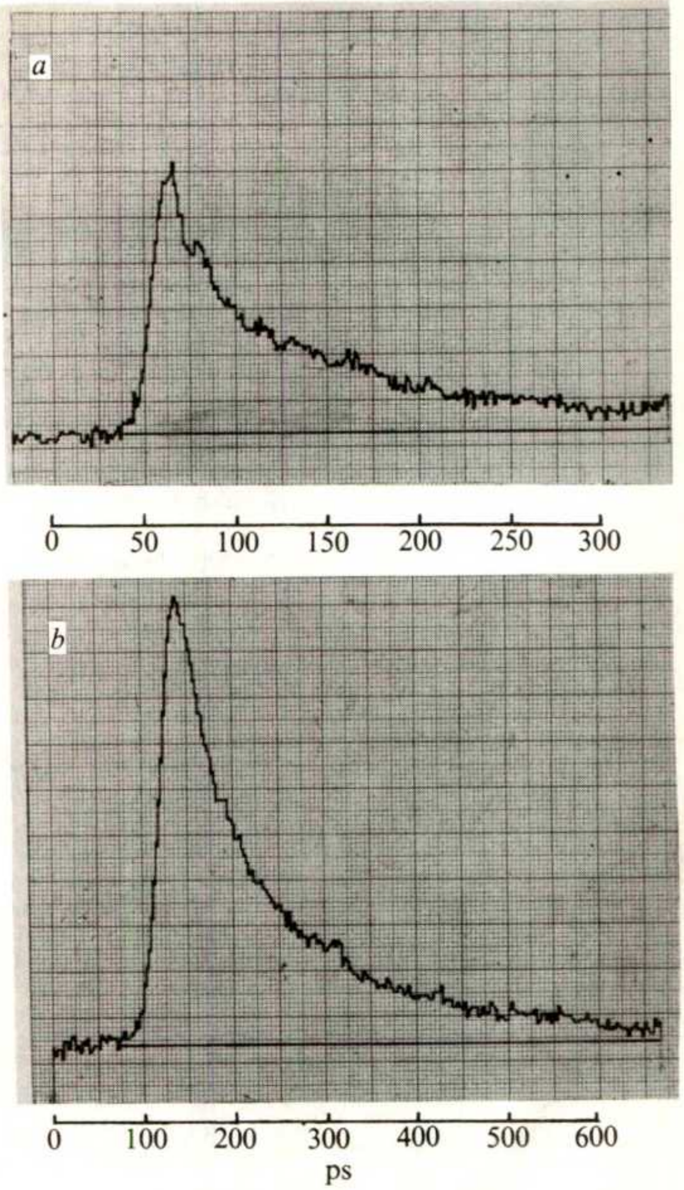


Fig. 2 Fluorescence decay of chloroplasts. *a*, Spinach chloroplast; *b*, spinach chloroplast and dithionite.

Table 2 Fluorescence yields of dithionite-treated chloroplasts

	τ (ps)	*f*	φ (calculated)		(measured) φ
PS I	130	0.82	0.0056	0.020	0.024
PS II	1,450	0.18	0.014		

photosystem I, and subscript II refers to the same quantities for system II; τ_0 is 19 ns, the natural lifetime of chlorophyll *a* (ref. 13). It will be seen from Table 2 that the calculated yield of total fluorescence agrees well with our direct measurements for the same chloroplasts.

It should be noted that the ratio, *f*, of 4.5, determined from the initial fluorescence intensities of the two decays, involves the accessory pigment absorption and measures the ratio of chlorophyll molecules excited in each system rather than the ratio of unexcited chlorophyll molecules. It refers to the excitation wavelength of 530 nm. The ratio is higher than has been predicted from measurements of the quantum yield of photosynthesis at this wavelength[14,15] and casts doubt on the feasibility of distinguishing photosystems I and II on the basis of their lifetimes.

Kollman, Shapiro and Campillo[7] have attributed the short lifetimes of chlorophyll fluorescence *in vivo* to concentration quenching, on the grounds that similar lifetimes are found in concentrated solutions of chlorophyll. Such an interpretation would be inconsistent with the operation of efficient photosynthesis and, although concentration quenching, probably of a special type which we discuss elsewhere, accounts for the lifetimes of fluorescence in concentrated solutions of chlorophyll *in vitro*, it cannot be important in an efficient photosynthetic system. We have considered one other possible cause of quenching of excited chlorophyll molecules in the *in vivo* measurements reported here—the intensity during measurement is so high that a significant fraction of the chlorophyll molecules is excited. Singlet-singlet annihilation could become important in these conditions, and possibly singlet–triplet annihilation involving triplets formed in earlier pulses. We therefore carried out the measurements on porphyridium over a tenfold range of intensities; the decay rates of fluorescence were unaffected by the intensity change and we conclude that exciton interactions are unimportant in the conditions of our experiments. In some cases, the initial rate, immediately after the flash, was somewhat faster than that reported in Table 1 but this would be expected from the time-dependent term in the theory of excitation diffusion (see ref. 16).

The lifetimes we have recorded are a direct measure of the average time taken for an absorbed photon to be trapped at the reaction centre in the conditions of our experiments.

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Fragmentation of benzylpenicillin after interaction with the exocellular DD-carboxypeptidase-transpeptidases of *Streptomyces* R61 and R39

THE killing target of penicillin in bacteria is a membrane-bound transpeptidase which catalyses peptide cross linking during wall peptidoglycan synthesis[1,2]. *Streptomyces* R61 and R39 excrete during growth DD-carboxypeptidase-transpeptidase enzymes[3,4] which seem to be soluble forms of the corresponding membrane-bound transpeptidases[5]. The exocellular enzymes (E) convert penicillin (I) in to a chemically altered and biologically inactive compound (X)[6,7]. Kinetically, the simplest mechanism[5] for the conversion of I into X is

$$E + I \overset{K}{\rightleftharpoons} EI \xrightarrow{k_3} EI^* \xrightarrow{k_4} E + X$$

The first step, a rapid equilibrium process, leads to the formation of an equimolar and inactive enzyme–antibiotic complex EI. This complex isomerises into a modified complex EI* which, in turn, undergoes irreversible breakdown. If the experiment is carried out in conditions in which the enzyme is stable, the enzyme is reactivated and recovers its initial penicillin sensitivity. The breakdown of complex EI* is a slow process. At 37 °C and in 10 mM Na phosphate buffer, *p*H 7.0 (in which conditions the R61 enzyme is stable) the half life of the R61 enzyme–benzylpenicillin EI* complex is 80 min (ref. 7). At 37 °C and in 0.1 M Tris-HCl buffer, *p*H 7.7, containing 0.1 M NaCl and 0.05 M $MgCl_2$ (in which conditions the R39 enzyme is stable), the half life of the R39 enzyme–benzylpenicillin is 4,250 min (ref. 6). As the enzyme is reactivated during the process, no enzyme is irretrievably lost on reaction with penicillin and after several cycles of inactivation and reactivation, both the enzyme and the accumulated X

Fig. 1 Mass spectra of authentic phenylacetylglycine (*A*, molecular weight = 193) and X compound (*B*). ^{14}C-X (0.5 μeq) was dissolved in 100 μl of 6 N HCl and extracted, at room temperature, with 300 μl of ethyl acetate (yield of the extraction 60% in terms of d.p.m.). Mass spectrography was carried out on a sample of the extract containing 5 neq of ^{14}C-X and compared with that given by 1 μg of phenylacetylglycine. Spectra were recorded at 70 eV with an ionisation current of 60 μA. The sample was directly introduced (193 : molecular ion M^+).

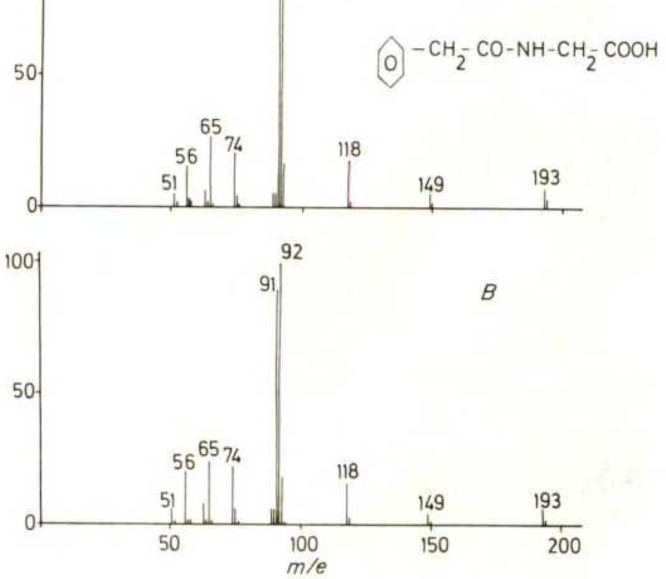

Table 1 R_f values of ^{14}C-X and phenylacetylglycine and of HCl-hydrolysed ^{14}C-X and phenylacetic acid, by cochromatography on Silica-Gel G thin-layer plates

Enzyme used for the preparation of ^{14}C-X	Solvent	R_f values of ^{14}C-X	Added phenylacetylglycine	HCl-hydrolysed ^{14}C-X	Added phenylacetic acid
R61	*a*	0.77	0.76	0.80	0.80
	b	0.71	0.70		
	c	0.40	0.40	0.56	0.55
R39	*a*	0.78	0.77		
	b	0.71	0.69		
	c	0.36	0.36		

Solvents: *a*, 1-butanol–H_2O–acetic acid–ethanol: 10:4:3:3 (v:v:v:v); *b*, water–1-butanol–acetic acid: 50:10:10 (v:v:v) upper phase; *c*, chloroform–methanol–acetic acid: 88:10:2 (v:v.v).

Note that the R_f values of benzylpenicilloic acid are 0.65 in solvent *a* and 0.55 in solvent *b*. Benzylpenicilloic acid is immobile in solvent *c*.

compound can be reisolated. This technique was applied to ^{14}C-benzylpenicillin (benzyl labelled) and the ^{14}C-X compound produced was isolated.

A solution of R61 enzyme (0.9 µmol; molecular weight 38,000) in 20 ml of phosphate buffer was supplemented at 37 °C with 0.72 µmol of ^{14}C-benzylpenicillin (0.7 mCi mmol^{-1}). Every 30 min, an additional 0.144 µmol of ^{14}C-benzylpenicillin was added until a total amount of 29 µmol of antibiotic was used. Filtration on Sephadex G-25 and elimination of phosphate by addition of acetone at 0 °C yielded 19 µeq of purified ^{14}C-X. Similarly, a solution of R39 enzyme (11.7 nmol; molecular weight 53,000) in 100 µl of Tris-NaCl-$MgCl_2$ buffer was supplemented with 14.5 nmol of ^{14}C-benzylpenicillin (25 mCi mmol^{-1}) and the mixture was incubated for 51 h at 37 °C. Filtration on Sephadex G-25 yielded 4 neq of ^{14}C-X.

The ^{14}C-X obtained with the R61 enzyme was identified as ^{14}C-phenylacetylglycine on the following grounds. (1) Acid hydrolysis with 6 N HCl at 120 °C yielded glycine (yield 100% with the amino acid analyser and 76% after transformation into dinitrophenylglycine) and ^{14}C-phenylacetic acid (as revealed by thin-layer chromatography on Silica-Gel plates using two solvents; Table 1). (2) ^{14}C-X and authentic phenylacetylglycine exhibited the same R_f values by thin-layer chromatography on Silica-Gel plates using three solvents (Table 1). (3) A mixture containing a few neq of ^{14}C-X and a 1,000-fold excess, on a molar basis, of non-radioactive phenylacetylglycine was submitted to three successive crystallisations from ethylacetate. Crystals thus obtained exhibited constant specific radioactivities. (4) The mass spectrum of ^{14}C-X was identical to that given by phenylacetylglycine (Fig. 1). (5) Both ^{14}C-X and phenylacetylglycine, esterified with CH_2N_2, had the same retention time (6 min) by gas–liquid chromatography (at 200 °C, with H_2 and N_2 flow rates of 2 and 30 ml min^{-1}, respectively, and by using a capillary SP1000 column of length 20 m). Similarly, the ^{14}C-X compound obtained with the R39 enzyme was also characterised as ^{14}C-phenylacetylglycine by cocrystallisation and cochromatography with authentic phenylacetylglycine.

We conclude, therefore, that by interacting with benzylpenicillin, both R61 and R39 exocellular enzymes split the antibiotic molecule and that one of the fragments is phenylacetylglycine. Evidently the methylene carbon atom of the glycine residue must arise from C_6 of the penicillin nucleus and, presumably, the C_7 is retained as the glycine carboxyl group (Fig. 2). The fate of the thiazolidine nucleus of the penicillin molecule has not yet been determined. Chemically, benzylpenicillin methyl ester can be degraded to methyl D-5,5-dimethyl-Δ^2-thiazoline-4-carboxylate in trifluoroacetic acid. The N-phenylacetylglycyl fragment was isolated by conversion to its N-benzylamide[8]. A possible mechanism for the action of the *Streptomyces* enzymes would be that the initial event is a rupture of the β-lactam amide bond (Fig. 2; arrow *a*) with attachment of C_7 to some active group in the enzyme By a process of β elimination, in which functional groups of the enzyme could participate, the link between C_5 and C_6 would become a double bond and further degradation would result in removal of the thiazolidine moiety (Fig. 2; arrow *c*). Release of the phenylacetylglycine would then occur. There may be other possible mechanisms and the problem is being studied at present.

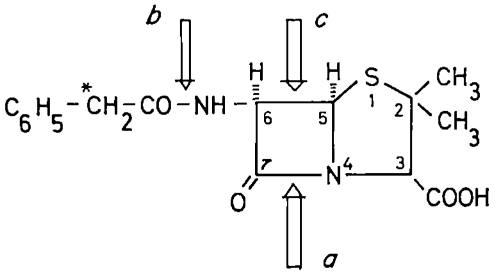

Fig. 2 ^{14}C-benzylpenicillin. *a*, Site of action of penicillinase (reaction product benzylpenicilloic acid); *b*, site of action of amidase (reaction product 6-aminopenicillanic acid); *c* and *a*, possible sites of action of the DD-carboxypeptidase-transpeptidases of *Streptomyces* R61 and R39 (reaction product phenylacetylglycine + unknown). *, [^{14}C].

Whatever the mechanism, however, our studies point to the great importance of the nature of the substituents on C_6 of the penicillin molecule. Substitution at C_6 drastically reduces the activity of the β-lactam antibiotics in all cases tested; 6-methoxypenicillin derivatives, however, remain better inhibitors than the corresponding 6-methyl derivatives[9]. These studies may also be related with the observations of Schmid and Plapp[10] that binding of penicillin to *Proteus mirabilis* was inhibited by phenylacetylglycine and that in the presence of this compound, formation of sphaeroplasts was prevented. Experiments have shown that the extracted and partially purified membrane-bound transpeptidases from *Streptomyces* R61 and *S. rimosus*[5] also perform fragmentation of benzylpenicillin with formation of phenylacetylglycine (M. Leyh-Bouille, J. Dusart and J. M. Ghuysen, unpublished). Moreover, penicillin is known to be degraded into a biologically inactive compound by interacting with the membrane-bound DD-carboxypeptidases of various *Bacillus* spp.[11]. The fact that the membrane-bound targets of penicillin act as antibiotic-degrading compounds is important even if the overall process is slow. It leads to a better understanding of the phenomena of resistance which cannot be explained on the basis of production of either penicillinase or amidase (Fig. 2; arrows *a* and *b*, respectively). It may also be relevant to the necessity for the continuous supply of antibiotic during penicillin therapy.

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Scotopic and photopic dark adaptation of the b wave in isolated rat retina

A SLOW form of rod dark adaptation lasting tens of minutes has been observed in isolated retinae[1–5], including those of the rat[6,7], following exposures to intense lights which bleach significant proportions of the rod pigment. Yet in the isolated rat retina in similar circumstances it has been reported[8,9] that the cells which produce the b wave of the electroretinogram adapt within minutes. Since this adaptation is finished before any of the slow thermal reactions of rhodopsin bleaching reach completion, a 'neural' basis for the phenomenon has been suggested. Experiments[10] have, however, confirmed earlier work[11] showing that there is more than one pigment in the rat. This provides an alternative explanation for the fast adaptation of the rat b wave. We have been able to demonstrate that during the fast adaptation the dominant input to the cells producing the b wave comes from the non-rhodopsin pigment system. We have also found conditions in which the b wave shows the slower form of adaptation. Then, the dominant input to the b-wave cells is from a rhodopsin-based mechanism.

Figure 1*a* shows that following the extinction of an intense light which bleaches 70% of, the rhodopsin in the retina, a very rapid adaptation can be seen in the b-wave threshold measurements, a similar phenomenon to that described previously[6,8,9]. From about 6 min the threshold remains stable, except for a very slight decline in the preparation towards the end of 30 min. Frank and Dowling[8] measured thresholds for periods of up to 1 h after a 50% bleach and also reported a stabilisation of the threshold What is new in the present study is that we have demonstrated a permanent spectral shift towards the red in b-wave sensitivity following the light exposure The intensities of the red and blue lights have been equated for their effect on the b wave before the exposure, when, presumably, rhodopsin rods provided the dominant input to the b-wave cells. After the light exposure, the threshold intensities are no longer equal This means that either bleach-increments thresholds for the rat b wave with lights of different wavelengths reaches the rhodopsin molecules, or the b-wave cells are controlled by a non-rhodopsin system Our results are analogous to those of Green[10] who studied increment thresholds for the rat b wave with lights of different wavelengths He too observed a shift in spectral sensitivity towards the red for intense backgrounds, and since this occurred with flashed backgrounds which bleached insignificant amounts of pigment, he interpreted the shift in terms of a transition from scotopic to photopic control of the b wave. It is plausible to interpret our results in the same way. Our conclusion, then, is that the very fast adaptation which we and others have described, reflects processes in a photopic mechanism with a pigment more sensitive to red light than rhodopsin.

Although the b wave shows fast adaptation, the PIII response (the slow component following the b wave and having the opposite polarity) shows the slow adaptation which we have previously reported and which we believe may be linked with the formation of retinol and free opsin at the end of the rhodopsin bleaching sequence[6,7]. PIII, as

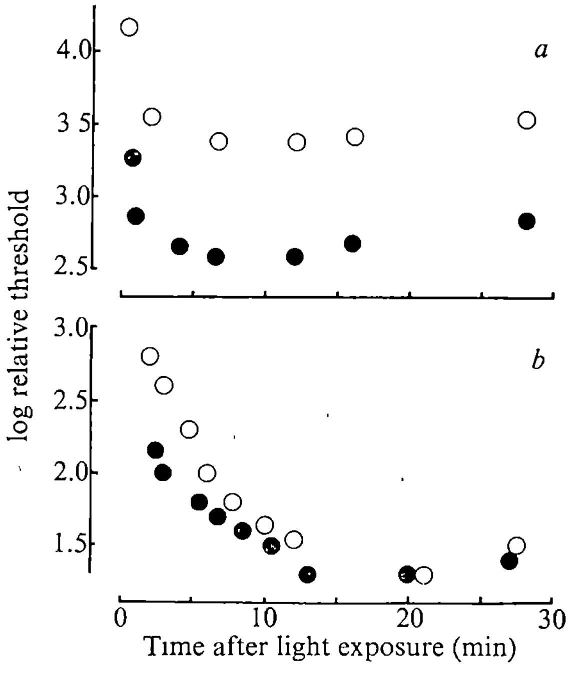

Fig. 1 Adaptation of the b-wave threshold for blue (○) and red (●) stimuli following light exposures which removed 70% (*a*) and 18% (*b*) of the rhodopsin from the dark-adapted retinae. Shimadzu interference filter, $\lambda_m = 482$ nm, used for blue light; Kodak, Wratten 25 filter for red light. b Waves in the range 5–30 μV were elicited from the dark adapted retinae with 50-ms blue and red flashes (frequency response of recording system 0.1–65 Hz). Estimates could thus be made of the calibrated neutral filters required with each type of flash to produce a criterion b wave. The retinae were then exposed to 1 min (*a*) or 25 s (*b*) of unattenuated blue light. At various times following the extinction of the bleaching light, measurements were made of the neutral density values required to elicit criterion b waves. Each of these values was subtracted from the pre-bleach value to provide the data plotted above. Apparatus and preparation details have been given previously[6]. The solution used for perfusion had the following composition (mmol dm^{-3}): NaCl, 119; KCl, 3.4; $NaHCO_3$, 21; NaH_2PO_4, 0.1; Na_2HPO_4, 0 8; $MgSO_4$, 2.4; $CaCl_2$, 2.0; and glucose, 28. It also contained 4% rabbit serum It was bubbled with O_2–CO_2 (95:5) to give a *p*H between 7.6 and 7.9, and its temperature at the retina was 30.5±0.5 °C.

measured here, is largely glial[12] and may have little to do with the signals sent by receptors to other neurones. The rat b wave on the other hand, though it too may have a glial origin[13], is a good index of ganglion cell activity[14]. In our earlier study[6], we therefore questioned how relevant the slow form of PIII adaptation was to understanding the adaptation processes of the rat visual system. Figure 1*b* shows, however, that the slow form of dark adaptation can also be seen in the rat b wave, provided the amount of rhodopsin bleached is relatively small. Although there is some shift in spectral sensitivity towards the red during the initial phase of adaptation, the final post-bleach spectral sensitivity is the same as that before the bleach. We conclude, therefore, that the slow form of adaptation is a property of rods containing rhodopsin. It seems to be very like the slow adaptation seen in the rods of other isolated retinae[1–5]. As Green[10] found in his study on the rat, PIII is always dominated by the rhodopsin-based mechanism. The b wave (and presumably the higher order neurones) will also reflect rod phenomena including slow dark adaptation, provided small amounts of rhodopsin are bleached and the elevation of the threshold is relatively small. On the other hand, for larger bleaches, a photopic mechanism comes to control the b wave. For the living eye and for small pigment bleaches of the isolated retina, this may be transient, but for large bleaches of the isolated retina, the photopic input to the b-wave cells becomes a permanent feature.

These conclusions have implications in the way previous investigations of the rat b wave are interpreted. For example, it has been claimed that a general relationship of the form:

$$\text{log threshold} \propto \text{fraction of pigment bleached}$$

underlies vertebrate dark adaptation. Work on the rat b wave represents one of the key pieces of evidence supporting this relationship (for a review, see ref. 15). The present study, however, makes it clear that many of the threshold measurements on the b wave in adaptation experiments reflect photopic activity and should not be correlated with the state of rhodopsin. Thus, the nature of the relationship between rhodopsin-based responses and the state of the pigment needs to be reconsidered.

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Inability of the cat to desaturate essential fatty acids

MOST vertebrate species require some dietary source of essential fatty acids (EFAs)[1,2]. A wide range of naturally occurring polyunsaturated fatty acids have been shown to exhibit EFA activity, but they can be classified into two homologous series characterised by the position of the terminal double bonds relative to the methyl (omega) carbon atom. The ω6 series of EFAs all have double bonds in the ω6 and ω9 positions, the ω3 series in the ω3, ω6 and ω9 positions. The metabolic interrelationships of the members of each series are shown in Fig. 1. The naturally occurring 18-carbon parent compounds for each series are linoleic acid (18:2ω6) and linolenic acid (18:3ω3) and EFA requirements are usually stated in terms of either or both of these parent EFAs (p-EFAs)[1,3].

Table 1 Fatty acid analyses of diets fed*

Fatty acid	Control	Diet SBOL	SSO
10:0+12:0+14:0	4.1	7.8	6.7
16:0+16:1	27.5	10.3	7.8
18:0	13.5	13.7	1.7
18:1	36.9	19.4	17.4
18:2ω6†	7.2	40.8	63.6
18:3ω3†	0.9	16.8	0.9
20:0 +20:1	1.9	—	—
20:2ω6‡	0.1	—	—
20:3ω6‡	0.1	—	—
20:4ω6‡	0.5	—	—
20:5ω3‡	2.3	—	—
22:4ω6‡	0.1	—	—
22:5ω6‡	0.1	—	—
22:5ω3‡	0.2	—	—
22:6ω3‡	0.4	—	—
Total p-EFAs	8.1	57 6	64.5
Total d-EFAs	3.8	0.0	0.0

*As g per 100 g total fatty acids.
†p-EFA.
‡d-EFA.
—, Indicates compound not detected (limit of detection about 0.04 g per 100 g total fatty acids).

Animal experiments, however, indicate that the higher homologues of the p-EFAs are the physiologically essential compounds[1]. The dietary importance of the p-EFAs probably results solely from their role as precursors of these higher homologues, the derived EFAs (d-EFAs). Studies in the rat have shown that the desaturation steps involved in the production of d-EFAs do not always proceed at an optimal rate, being relatively easily inhibited by dietary or metabolic factors[4,5]. We report here the results of dietary studies on the domestic cat which indicate that this species may lack the ability to further desaturate polyunsaturated fatty acids, and therefore exhibit a specific requirement for lipid of animal origin.

Cats (*Felis catus* L., domestic cross) were bred in this laboratory and when they weighed 0.8–1.7 kg were placed on purified experimental diets which avoided the use of animal lipids likely to be rich in d-EFAs. The two experimental diets used were based on casein (550 g kg^{-1}) and added oil (150 g kg^{-1}). This oil was safflower seed oil in the SSO diet; and a 5:1 (w/w) mixture of soybean oil and linseed oil in the SBOL diet. Both diets contained high levels of cholecalciferol (2.5 mg kg^{-1} diet) but otherwise were designed to satisfy the known nutrient requirements of the cat[6,7]. The control diet was a mixture of proprietary meat- and fish-based cat foods. It contained, on a dry weight basis, 450 g kg^{-1} crude protein and 180 g kg^{-1} lipid, and has proved satisfactory for maintaining our cat colony. The fatty acid composition of the diets is shown in Table 1. One male and one female were fed the SSO diet, two males and three females the SBOL diet and two males and two females the control diet for an average period of fifteen months (range 12 to 18 months).

The fatty acid composition of phosphoglycerides of plasma and red blood cells was examined by standard techniques[8]. The results obtained for plasma choline phosphoglycerides (CPGs) are shown in Table 2; ethanolamine phosphoglyceride and CPGs of red blood cells showed parallel changes. The tissue levels of d-EFAs were much reduced in experimental animals, only arachidonic acid (20:4ω6) and 11,14-eicosadienoic acid (20:2ω6) being present in detectable amounts. That these low levels occur in spite of the presence of very high amounts of

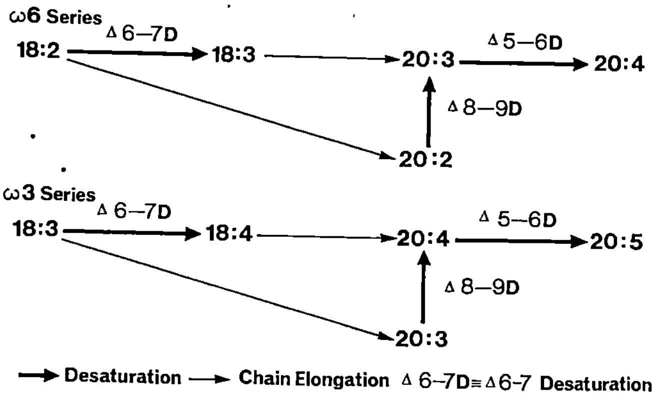

Fig. 1 Metabolism of the essential fatty acids (omitting the 22-carbon fatty acids).

18:2ω6 in diet and tissue lipids, suggests that the experimental animals are unable to chain elongate or desaturate 18:2ω6. Since 20:2ω6 is not present in the diet, nor in the plasma CPG of normal cats, its presence in experimental animals demonstrates that chain elongation did occur. Studies in the rat have shown that the preferred pathway for the metabolism of 18:2ω6 involves a Δ6–7 desaturation to 18:3ω6 (Fig. 1). The chain elongation of 18:2ω6 to 20:2ω6 is relatively unimportant unless desaturation is impaired, therefore the detection of significant quantities of 20:2ω6 and the virtual absence of desaturation products of 18:2ω6 both imply that the Δ6–7 desaturase activity is lacking. Moreover, the absence of 8,11,14-eicosatrienoic acid (20:3ω6) in spite of the high levels of 20:2ω6 is indicative of the absence of Δ8–9 desaturase activity in the cat. Sprecher[9] has recently reported that Δ8–9 desaturase activity is also absent in the rat.

Animals fed the SBOL diet seemed to have higher levels of 20:4ω6 than the two animals receiving SSO diet, but in our routine gas liquid chromatography system 20:4ω6 cochromatographs with 11,14,17-eicosatrienoic acid (20:3ω3). By the use of a 50-m capillary column we were able to separate these components and show that the 20:4ω6 peak contained about 40% 20:3 ω3. We failed to detect 20:3ω3 in the 20:4ω6 peak of animals fed SSO. We conclude that the plasma CPG levels of 20:4 ω6 were about the same in the two experimental groups and that the presence of 20:3ω3 confirms the absence of Δ6–7 and Δ8–9 desaturase activity with ω3 fatty acids as a substrate.

The unknown fatty acid detected in plasma CPG of animals fed all experimental diets has not yet been identified fully. We have established that it is a 20-carbon straight chain fatty acid and that it does not belong to the ω3, ω6 or ω9 series. Investigations into its structure are continuing.

Table 2 Fatty acid composition of plasma choline phosphoglycerides

	Control (n = 4)	SBOL fed (n = 5)	SSO 1	SSO 2
16:0+16:1	14.8±0.05	10.2±1.06	9.5	6.9
17:0	0.7±0.06	0.2±0.14	0.5	—
18:0	29.9±0.64	26.3±1.18	23.7	27.0
18:1	13.1±0.85	9.5±0.89	10.0	6.0
18:2ω6	11.8±0.86	45.6±1.25	47.5	49.8
18:3ω3	0.3±0.11	2.6±0.29	0.4	0.5
20:0+20:1	0.6±0.12	0.7±0.04	1.6	0.3
20:2ω6	—	2.2±0.24	4.9	6.2
Unknown	—	1.5±0.25	1.6	3.0
21:0	0.1±0.00	—	—	—
20:3ω6	1.1±0.15	—	—	—
20:4ω6+20:3ω3	14.3±1.05	0.9±0.14	0.3	0.3
20:5ω3	8.0±1.63	—	—	—
22:4ω6	0.9±0.34	—	—	—
22:5ω3	0.8±0.14	—	—	—
22:6ω6	4.1±0.39	—	—	—
Total p-EFA	12.1	48.2	47.9	50.3
Total d-EFA	29.2	3.1	5.2	6.5

Fatty acids as g per 100 g total fatty acids as mean (± s.e.m. where appropriate).

—, Indicates compound not detected (limit of detection about 0.04 g per 100 g fatty acids).

Animals fed the experimental diets showed a varied clinical picture. All were listless, however, and had dry staring coats and severe dandruff. They grew poorly and seemed to be severely affected by infections and stress. Histological examination of their livers showed much fatty infiltration and parenchymal disorganisation. Both male and female animals became behaviourally infertile on the experimental diets, testes were underdeveloped and vaginal smearing failed to demonstrate the occurrence of oestrous cycles. We cannot explain these effects as a result of the level of dietary vitamin D, and, although limited by the lack of a suitable control group, there seems to be a similarity in this clinical picture with that caused in other species by feeding diets lacking in EFA activity[1,10]. Few attempts have been made to feed cats defined diets for prolonged periods, but these have resulted in reduced growth and failure to breed[11]. It may well be that deficiency of d-EFAs in purified diets caused these defects.

So far, one female SBOL animal has been returned to the control diet. This caused a rapid and sustained change in the fatty acid pattern of plasma CPGs (Table 3) and a marked improvement in clinical state. The animal rapidly gained weight (0.5 kg in 32 d), its coat became thicker and glossier and the dandruff disappeared. Its behaviour altered—the animal becoming more friendly and less restless. On day 32 of refeeding the animal came into oestrus and was successfully mated: after an uncomplicated pregnancy she gave birth to, and suckled, four normal young.

Table 3 Changes in essential fatty acids of plasma CPG in a SBOL cat refed control diet

Diet	SBOL	Control		
Days on control diet	0	3	11	32
	Fatty acids g per 100 g total fatty acid			
18:2ω6	48.3	34.6	24.4	13.9
18:3ω3	2.6	0.9	0.6	0.1
Unknown	0.8	1.0	0.8	0.1
20:2ω6	1.8	0.7	0.3	—
20:3ω6	—	0.6	0.8	0.9
20:4ω6+20:3ω3	0.5	4.8	9.9	8.8
20:5ω3	—	1.6	3 4	15.0
22:5ω3	—	1.0	1.8	0.8
22:6ω3	—	1.5	4.0	5.2
Total p-EFA	50.9	35.5	25.0	14.0
Total d-EFA	2.3	10.2	20.2	30.7

—, Indicates compound not detected (limit of detection about 0.04 g per 100 g total fatty acid).

Our observations clearly indicate a lack of Δ6–7 and Δ8–9 desaturase activity in the cat. Preliminary results (J.R. and A.J.S., unpublished) indicate that the Δ5–6 desaturase activity is lacking so that conversion of 20:3ω6 to 20:4ω6 cannot occur.

We conclude that the EFA requirement of the cat can only be satisfied by a diet containing d-EFAs, regardless of the dietary levels of p-EFAs. This phenomenon has not previously been described in any animal species; however, a d-EFA requirement would explain an observation that animal lipid was a necessary dietary component for optimal health in mink[12]. Classically, EFA deficiency is identified by an increase in tissue levels of 5,8,11-eicosatrienoic acid (20:3ω9), a non-essential derivative of oleic acid synthesised by animals fed diets deficient in EFA. We did not observe 20:3ω9 in our animals, a further indication of the absence of Δ6–7 and Δ5–6 desaturases. We postulate that a total EFA deficiency could be induced in the cat without significant levels of 20:3ω9 being produced.

The absence of desaturase activity in the cat makes it a particularly good experimental model for research on prostaglandins and essential fatty acid metabolism. The possibility that other species adapted to an omnivorous or carnivorous diet might show a d-EFA requirement also deserves investigation.

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Folate-dependent 1-carbon transfer to biogenic amines mediated by methylenetetrahydrofolate reductase

IT has been suggested that N-methylation of biogenic amines is involved in the development of certain schizophrenic disorders[1,2]. Interest in this transmethylation hypothesis has been boosted by the reported identification of a mammalian N-methyltransferase ("dopamine methyltransferase"[3]) which uses as methyl donor N^5-methyltetrahydrofolate (MTHF) rather than S-adenosyl methionine[3–5]. Further studies have demonstrated that the products of the incubation of catecholamines or indoleamines with MTHF and the transferase were not N-methylated amines but tetrahydroisoquinoline or tetrahydro-β-carboline alkaloids, respectively[6–10]. These compounds can be formed readily by the condensation of formaldehyde with the amines and it has therefore been postulated that the enzyme originally identified as a methyltransferase may in fact act to release formaldehyde from MTHF[6–10]. We provide evidence that the enzyme methylenetetrahydrofolate reductase (EC 1.1.1.68) is responsible for the reported production of formaldehyde from MTHF.

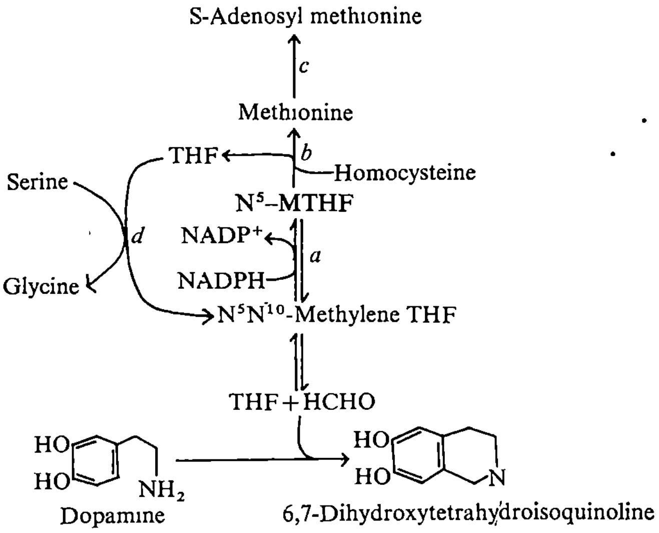

Fig. 1 Folate metabolism and tetrahydroisoquinoline synthesis. The enzymes involved are: *a*, methylenetetrahydrofolate reductase (EC 1.1.1.68); *b*, N^5-MTHF homocysteine methyltransferase (EC 2.1.1.13); *c*, methionine adenosyltransferase (EC 2.5.1.6); *d*, serine hydroxymethyltransferase (EC 2.1.2.1) The dissociation of methylenetetrahydrofolate to tetrahydrofolate and formaldehyde and the reaction of formaldehyde with dopamine are presumed to be non-enzymic.

Methylenetetrahydrofolate reductase catalyses the NADPH-dependent reduction of N^5,N^{10}-methylenetetrahydrofolate to MTHF (see Fig. 1). In the reverse direction, in the presence of a suitable oxidant, MTHF is converted to methylenetetrahydrofolate which may dissociate to tetrahydrofolate and formaldehyde[11]. The release of formaldehyde from MTHF may be used as an assay procedure, but the reductase may also be assayed spectrophotometrically in the direction of MTHF formation[11]. In this work both assays have been used and dopamine methyltransferase was measured radiometrically as described by Laduron[3].

High activities of both methylenetetrahydrofolate reductase[11] and dopamine methyltransferase[5] are reported to occur in liver, and this tissue was used for most of the studies reported here. The reductase was purified to a specific activity of 0.1 U mg^{-1} as described by Kutzbach and Stokstad[11] and the activities of the reductase and of dopamine methyltransferase copurified during this procedure. The product was further fractionated by gel filtration or isoelectric focusing.

Gel filtration was performed on a calibrated Sephadex G-200 column and the eluate was assayed for methylenetetrahydrofolate reductase and dopamine methyltransferase. Both activities coincided in a single symmetrical peak with an elution volume corresponding to an apparent molecular weight of 170,000–190,000 (Fig. 2*a*). Both methylenetetrahydrofolate reductase and dopamine methyltransferase were recovered in a single peak corresponding to an

Table 1 Effect of various additives on the activities of methylenetetrahydrofolate reductase and dopamine methyltransferase

Additive	Methylenetetrahydrofolate reductase activity (%)	Dopamine methyltransferase activity (%)
None	100	100
FAD (2×10^{-6} M)	410	320
DOPAC (1×10^{-2} M)	47	58
S-Adenosyl methionine ($1 6\times10^{-4}$ M)	< 10	< 10
S-Adenosyl methionine (1.6×10^{-4} M) +S-adenosyl homocysteine (1.6×10^{-4} M)	54	100

Methylenetetrahydrofolate reductase was purified from pig liver as described in the text and its activity was measured spectrophotometrically at 340 nm as described by Kutzbach and Stokstad[11]. Dopamine methyltransferase was assayed radiometrically as described by Laduron[3].

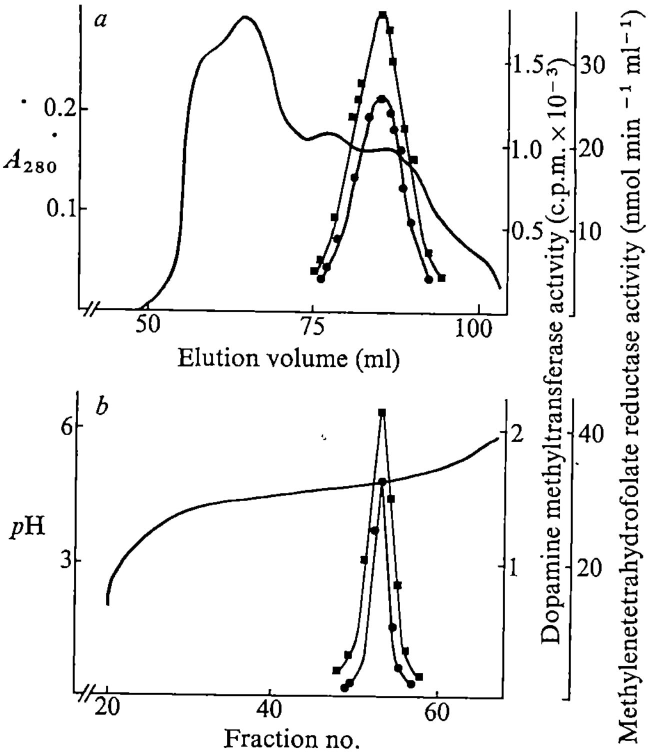

Fig. 2 *a*, Isolation of methylenetetrahydrofolate reductase by gel filtration on Sephadex G-200. 10 mg methylenetetrahydrofolate reductase (specific activity, 0.1 U mg^{-1}) purified from pig liver by the method of Kutzbach and Stokstad[11] was applied to a Sephadex G-200 column (1.5×95 cm) equilibrated in 10 mM potassium phosphate buffer, *p*H 7.5 containing 1 mM EDTA and 1 mM mercaptoethanol. Fractions were assayed for protein (A_{280}) and for methylenetetrahydrofolate reductase (●) and dopamine methyltransferase (■) activities as described in the text. *b*, Isoelectric focusing of methylenetetrahydrofolate reductase. 10 mg methylenetetrahydrofolate reductase was electrofocused at 4 °C in a 110 ml LKB column using ampholine, *p*H 3.5–5.0, in a 20–60% glycerol gradient. When electrofocusing was complete the column was drained and 2 ml fractions were collected. The *p*H value and the activities of methylenetetrahydrofolate reductase (●) and dopamine methyltransferase (■) of the fractions were measured. The recovery of enzyme activity was 50–60%.

isoelectric point of 4.8 (Fig. 2*b*). A similar result was obtained when dopamine methyltransferase, partially purified from bovine brain by the method of Laduron[4], was assayed by isoelectric focusing.

Active fractions from the gel filtration column were pooled and concentrated and used to investigate the effects of several additives on enzyme activity. The assay procedure for methylenetetrahydrofolate reductase routinely includes the addition of 2 μM FAD which produces a fourfold stimulation of the rate observed in the absence of the flavin coenzyme[11] and, correspondingly, the activity of dopamine methyltransferase was shown to be significantly increased in the presence of FAD (Table 1) 3,4-Dihydroxyphenylacetic acid (DOPAC) has been reported to inhibit the transferase[12]. We observed that 10^{-2} M DOPAC inhibited both the transferase and methylenetetrahydrofolate reductase activities to similar extents (Table 1).

Methylenetetrahydrofolate reductase is the initial enzyme in the pathway leading to the synthesis of S-adenosyl methionine (see Fig. 1). The reductase is allosterically inhibited by S-adenosyl methionine and this inhibition is relieved by S-adenosyl homocysteine[11]. As shown in Table 1, 1.6×10^{-4} M S-adenosyl methionine inhibited both the reductase and the transferase to similar extents and this inhibition was indeed reversed by S-adenosyl homocysteine.

The evidence presented here therefore supports the hypothesis that methylenetetrahydrofolate reductase and dopamine methyltransferase represent two activities exhibited by a single enzyme species. It is noteworthy that the regional distribution of these two activities in brain have previously been shown to be similar[13,14]. The mechanism of 1-carbon transfer to biogenic amines by the reductase *in vitro* would seem to involve oxidation of MTHF to methylenetetrahydrofolate and subsequent non-enzymic dissociation of formaldehyde from the folate derivative (Fig. 1). It is, at present, uncertain whether mediation in the synthesis of tetrahydroisoquinoline and tetrahydro-β-carboline alkaloids represents a physiological activity of this enzyme. Both the equilibrium of the reaction[11] and the NADPH–NADP$^+$ ratio in the cytosol[15] would favour MTHF synthesis rather than the reverse reaction involving formaldehyde release. Furthermore, free formaldehyde may also bind to tissue protein[16] or be oxidised by formaldehyde dehydrogenase[17]. The feedback inhibition of methylenetetrahydrofolate reductase by S-adenosyl methionine also supports the concept that the physiological function of this enzyme is in the ultimate synthesis of S-adenosyl methionine rather than the metabolism of biogenic amines. Since the formation of pharmacologically active alkaloids[18] from MTHF and amines may be detected *in vitro*, however, it would seem to be important to investigate their possible formation *in vivo*.

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Errata

In the Matters Arising contribution "Development of visual acuity and the sensitive period" by J. T. Flynn, D I Hamasaki, T. E. Flynn and M. Barricks (*Nature*, **257**, 337; 1975) the author of the original article quoted in ref 1 should be D. N Freeman and not R. D. Freeman.

In the article "Superior growth of the right gonad in human foetuses" by U. Mittwoch and D. Kirk (*Nature*, **257**, 791, 1975) the second sentence of the legend to Fig. 1 should begin: Bars represent the means of the . . . and not as printed.

matters arising

Lunar magnetism

In a recent series of papers, Runcorn[1,2] has maintained that the observation of a very small lunar surface dipole field ($\lesssim 0.05\gamma$) implies that there used to be a fairly strong interior lunar dipole moment ($\gtrsim 3.2\times10^9$ yr ago). He contends that if such a field had disappeared in the past 3.2×10^9 yr, the exterior field of the Moon would now be zero. This, he argues, is a direct result from potential theory.

I show, for a very simple model of the Moon, that if a primordial core magnetic field existed, it would give rise to a present day non-zero dipole external field.

Consider a uniformly magnetised core of radius a, embedded in a permeable mantle with outer radius b. The core magnetisation is $M_0 = M_0\hat{e}_3$ and the scalar potential of the magnetic field, H, satisfies $H = -\nabla\varphi(x)$, with $B \sim \mu H$ in the mantle. The boundary value problem is easily solved with the result.

$$\Phi_C(x) = \alpha r \cos\theta \tag{1}$$

$$\Phi_M(x) = (\beta r + \gamma/r^2)\cos\theta \tag{2}$$

$$\Phi_V(x) = \delta/r^2 \tag{3}$$

where C, M, and V refer to core, mantle, and vacuum, respectively; and

$$\begin{aligned} \alpha &= \beta + \gamma/a^3 \\ \beta &= -2(1-\mu)A \\ \gamma &= b^3(\mu+2)A \\ \delta &= 3\mu b^3 A \\ A &= 4\pi M_0 a^3/D \\ D &= (2\mu+1)(\mu+2)b^3 - 2a^3(1-\mu)^2 \end{aligned} \tag{4}$$

Now imagine that the magnetising currents in the core die out.

The magnetisation of the mantle in the absence of a core field is then

$$M(x) = \frac{(\mu-1)}{4\pi}\left[-\beta + \frac{2\gamma}{r^3}\right]\cos\theta\hat{e}_r + \left[\beta + \frac{\gamma}{r^3}\right]\sin\theta\hat{e}_\theta \tag{5}$$

The scalar potential, $\Psi(x)$, of the resulting field is

$$\Psi_C(x) = -\frac{2}{3}\frac{(\mu-1)}{a^3}(1 - a^3/b^3) \times \times \gamma r\cos\theta \tag{6}$$

$$\Psi_M(x) = \frac{(\mu-1)}{3}[\beta(a^3 - r^3) - -2\gamma(1 - r^3/b^3)]\frac{\cos\theta}{r^2} \tag{7}$$

$$\Psi_V(x) = \frac{(\mu-1)}{3}(b^3 - a^3)\beta\frac{\cos\theta}{r^2} \tag{8}$$

Equation (8) leads to a non-zero external dipole field. Runcorn's conclusion[1,2], that the external field is zero, is based on his assertion that the potential of the magnetising field has the form of equation (2), but with $\beta = 0$. Clearly, if $\beta = 0$ in equation (2), then $\Psi_V(x) = 0$. It must be emphasised that, using an internal magnetising field, the external field is in general non-zero after the core field has decayed to zero. The solution given by equations (6)–(8) is, in fact, a linear combination of the 'interior' and 'exterior' solutions discussed by Runcorn[2].

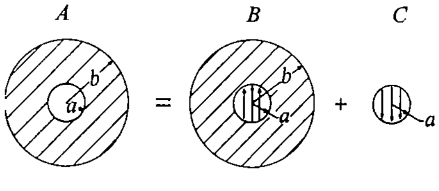

Fig. 1 The superposition of the magnetic fields. The field in A where there is no core magnetisation can be derived by subtracting the field of a uniformly magnetised sphere, radius a, (C) from that of the large uniformly magnetised sphere (B). See the equations for details.

The conclusion that the external field is not zero also follows from the linearity of the magnetostatic field equations. The solution of the problem with zero core field can be obtained from the solution with non-zero field, equations (1)–(4), by adding to the fields derived from equations (1)–(4), the field of a uniformly magnetised sphere of radius a, with magnetisation $M' = -M_0\hat{e}_3$. This is indicated schematically in Fig. 1. The resulting external field will clearly be a dipole of reduced strength. The fields resulting from such a superposition are in fact identical to those resulting from equations (7) and (8).

Stephenson *et al.*[3] note that Runcorn's result is strictly true only if the magnetic susceptibility of the mantle is very small, $\sim 10^{-4}$. Although it is clear that the exterior field is of higher order in $(1-\mu)$ than the fields in the other two regions, one must be cautious about arguing that it is therefore negligible. The permeability has been treated as though it were paramagnetic in this simple derivation, but it must, of necessity, be ferromagnetic. To my knowledge the ferromagnetic permeability of the Moon is not known. Dyal *et al.*[4] have, however, found a paramagnetic permeability $\mu \sim 1.01$, that already is larger than the value of $1 + 10^{-4}$ used by Stephenson *et al.*[3]. This larger value for μ implies the existence of ferromagnetic material[4], the properties of which are undetermined.

I acknowledge discussions with Dr N. F. Ness and J. D. Scudder.

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RUNCORN REPLIES—The theorem[1,2] which I proved is exactly correct as I stated it: that is, if a spherical shell of any thickness acquires permanent magnetisation, the intensity of which is proportional and parallel to a magnetising field of internal origin, which later disappears, its external field is zero. In the simpler proof[1], the magnetising field was assumed to be that of a centred dipole and I used the theorem to show that the new determination[3] of the present lunar dipole magnetic field as $< 1.3 \times 10^{18}$ gauss cm^3, or $< 0.05\ \gamma$ at the equator (essentially negligible), is very powerful evidence for the existence of an ancient lunar magnetic field of internal origin, which had been postulated to explain the natural remanent magnetisation discovered in the lavas returned by the Apollo missions[4], and effectively excludes an external agency unless it resulted in random magnetisation.

It was shown[2] that if the maximum magnetisation of the shell at its exterior is I, then, for the dipole case, there is within its inner boundary, a uniform field of intensity $4\pi\ I/3$ times the ratio of the volume of the shell to that of the unmagnetised inner sphere. If the depth of the Curie point isotherm is taken as 50 km, this ratio is 0.1, and if $I = 10^{-4}$ e.m.u. g^{-1} the uniform field is 10 γ.

I was aware that such a field, derived from the outer part of the magnetised shell, not only induces magnetisation in its interior, the point of Goldstein's communication, but would also have modified the magnetising field in which the rocks, cooling through the Curie point, acquired thermoremanent magnetisation. The resultant component of uniform magnetisation of the shell, increasing inwards, will give rise to an external dipole field, as will the departure of the Moon from sphericity, but I considered these effects and found them negligible[2]. Goldstein incorrectly quotes us in the last paragraph: we[6] stated that, for the theorem to be applicable, c, the magnetisation acquired in unit field, must be very small, of the order of 10^{-4}, not the magnetic susceptibility ($\mu - 1$) which even if it is 10^{-2}, as one analysis gives[7], produces a dipole field of much less than the observable limit. As the equatorial surface field of a shell, of uniform magnetisation I', is $4\ \pi\ I'/3$ multiplied by the ratio of the volumes of the shell to the sphere, this external field is about 0.01 γ for the Moon.

In devising a mathematical model to describe and understand the significance of experimental data, the scientist simplifies the physical situation by leaving out of consideration factors which are unimportant. Lyttleton[8] has lately reminded geophysicists of Eddington's description of the process. It is entirely legitimate for the mathematician to point out that the most general expression has not been used, but he must show quantitatively that with this added complexity, observations can be better fitted or the inferences to be drawn are thereby qualitatively changed; Goldstein does neither. But perhaps when the accuracy and reliability of magnetometers carried in spacecraft is improved by orders of magnitude, the further refinement of the theorem will be necessary. Goldstein may be drawing an analogy with the Earth—often misleading for lunar scientists—where the induced magnetisation of geological formations is usually of the same order as the permanent magnetisation.

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Effects of gallic acid on nitrosamine formation

OBSERVATIONS[1] on the catalytic effect of the naturally occurring polyphenol, chlorogenic acid, on the nitrosation of piperidine, may be supplemented by similar observations we have made during an investigation into the effect of gallic acid—an essential constituent of the gallotannins—on nitrosation of diethylamine. The possibility that such compounds could exert an influence on the *in vivo* formation of carcinogenic nitrosamines deserves careful attention.

Though early experiments[2] established the existence of an impeding effect by tannins on the formation of nitrosamines, more recent work shows that the amount of nitrosamine formed is dependent both on the *p*H and the concentration of the gallic acid.

We reacted concentrations of nitrite (0.075 M) and diethylamine (0.5 M) in buffered aqueous solutions, in the presence of different concentrations of gallic acid. Reaction was allowed to proceed for 30 min after which the solution was made alkaline to stop further reaction, and extracted with methylene chloride. The concentration of nitrosamine was determined using both ultraviolet spectrophotometry and gas chromatography. The concentrations of both the nitrite and the amine were chosen to give a convenient yield of nitrosodiethylamine for measurement of both systems. The relatively high concentration of amine allowed the use of a lower concentration of nitrite so that the competitive reaction of nitrite with gallic acid was not masked.

The results show (Fig. 1) that although gallic acid does exert a catalytic effect, it is extremely dependent on *p*H and is restricted to a narrow range in the region of *p*H 4. In addition, the balance between the retarding and catalytic effect is strongly dependent on the concentration of gallic acid, as illustrated by the fact that an increase in the formation of nitrosamine related almost linearly with a decrease in the concentration of gallic acid. Similar effects were also found when solutions of tannic acid were used in place of gallic acid.

Though this tends to support the views of Challis and Bartlett[1], the idea that a catalytic effect by natural phenols can lead to the *in vivo* formation of nitrosamines, should be considered with caution. Nitrosamine formation could be dependent on the variable *p*H conditions in the digestive system and on eating and drinking habits which could regulate the simultaneous concentrations of nitrite, tannins and nitrosatable amines that may have been ingested or formed in the system.

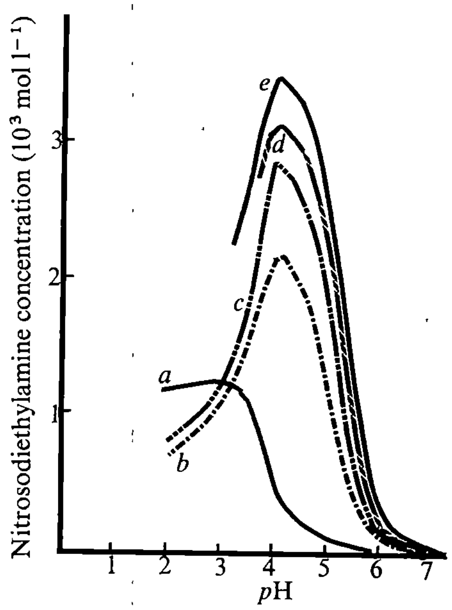

Fig. 1 Diagram demonstrating the increasing catalytic effect of gallic acid on nitrosodiethylamine formation with decreasing concentrations of gallic acid. Gallic acid concentration: *a*, 0; *b*, 0.0625 M; *c*, 0.0375 M; *d*, 0.025 M; *e*, 0.0125 M.

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reviews

Antibody molecule

R. R. Porter

The Antibody Molecule. (Immunology: An International Series of Monographs and Treatises.) By Alfred Nisonoff, John E. Hopper and Susan B. Spring. Pp. xiv+542. (Academic: New York, San Francisco and London, August 1975.) $34.50; £16.55.

THE capacity of those individuals who survive infectious diseases to become immune to further attack was recognised early in history, and for several hundred years attempts have been made to exploit this phenomenon in medicine. It is nearly one hundred years since Behring showed that the substances which are responsible are present in the blood of the immune animal and named them antibodies, but it is less than twenty years since advances in knowledge of protein chemistry made it possible to isolate and study the structure of these remarkable molecules. Their fascination lies in the ability of almost identical molecules to combine specifically with an apparently endless range of different substances and the recognition that this phenomenon is essential for the survival of all species of vertebrates. The growing realisation that a related phenomenon may be the basis of cellular recognition and therefore be an important feature of differentiation has put the antibody molecule in a key position in biological research. Here for the first time, the variable region of antibody molecules offers a chemical basis for one aspect of the infinite variety of living things. The genetic origin of this very wide range of protein structures found in all individuals remains a mystery, but one likely to be solved soon.

Even so, it is surprising to find in this book just how much information has been acquired in so short a time. The emphasis throughout the book is on the structure of the immunoglobulins, the proteins which carry the antibody activity. The chemical structures which define the division of the immunoglobulins into class and subclass, type and subtype, the different polymorphic forms, and the amino acid sequence changes found in the variable regions are all well described Most of this work is soundly based, and although much remains to be investigated sufficient detailed study has been carried out on the immunoglobulins of several species to provide a body of knowledge which is now the basis for present investigations on the genetics, the control of synthesis and cellular origins of immunoglobulin, and on many other aspects of immunity.

The authors have written an advanced monograph in which a mass of complex data has been presented in a well ordered and comprehensible manner. Sufficient detail is given to make this book an excellent source of reference for those working in the field, but it never obscures the main themes. It should be of equal value for the many biologists with a peripheral interest in the subject. The book has been published quickly: most of the X-ray crystallographic studies of the past year or two are described and a most informative coloured plate has been included of the model of the *Fab* fragment of a human myeloma protein. Enough experimental detail will be found to support the results described and to explain, for example, the discovery of the still somewhat obscure idiotypic specificities of antibodies.

Most of the work described is of immediate interest, but some, such as the early attempts to define the relative contribution of the heavy and light chain of antibodies to the structure of the combining site, have a dated flavour and already seem to have been superseded by subsequent knowledge of the chemistry and crystallographic studies of the variable regions. Succinct accounts are included of the brief histories of most of the topics discussed and are incomplete only in the overmodest absence of references to the senior author. Careful reading of the extensive bibliographies at the end of each chapter is necessary to appreciate how large a contribution to our knowledge of antibody structure has been made by Dr Nisonoff.

This book gives a clear account of our factual knowledge of the structure of antibodies and of its biological significance It is likely to remain of enduring value for some years to come.

Man and wildlife

Man and Wildlife. (Biology and Environment.) By L Harrison Matthews. Pp. 129+16 plates (Croom Helm: London, September 1975.) £4.95.

WE have been here many times before—from early man and increasing plunder of wildlife to population explosion and pollution—but this is a very readable addition to the considerable clutch of similar books.

The detailed effects of man's impact on wildlife (and vice versa) are discussed in convenient form. There are chapters on plants and topography, the sea, marine mammals, birds, insects, and other invertebrates, followed by one on "Conservation Profit and Loss" and a rather curious bibliography for further reading.

The authoritative and lively style is to be welcomed Not so, instances of what seems to be prejudice leading to dubious generalisation and emotive references out of tune with the character of the book As an example, "superficial" wildlife films on television as well as bird watchers with their "little" lists are castigated without acknowledgement that herein may lie the genesis of funds for holding open the conservation options for future generations— to purchase Wash and Lake Nakuru mud, and to pay for 'Operation Tiger' and education all over the world.

The Publishers' note on the wrapper expressing some hope for wildlife's future is clearly not endorsed by the author in his concluding chapter which omits adequate analysis of the conservation effort. Yet if we are to accept that all hope is lost at this stage, much of the *raison d'être* of civilised man is lost also.

Derek Barber

Cognitive aspects of reading

The Psychology of Reading. By Eleanor J. Gibson and Harry Levin. Pp. xii+630. (MIT: Reading, Massachusetts and London, 1975.) $18.95.

THE republication of Huey's *Psychology and Pedagogy of Reading* after sixty years of general reticence on the topic has been accompanied by a rush of modern treatments. This rebirth of interest in reading reflects not only the cunning propensity of psychology for crop rotation but also an increasing interest of cognitive psychologists in skilled and intelligent performance. At the same time social concern about literacy, especially in the US, has enabled the psychologist, like a modern Danaë, to be possessed by Zeus in a shower of research funds. This volume by Gibson and Levin presents the most comprehensive engagement to date of cognitive psychology with the problem of reading.

The book consists of three sections, the first of which reviews the basic psychological and linguistic principles which the authors believe relevant. These include perceptual learning, work on general, cognitive and especially language development, basic linguistics and some specialised material on writing systems, and finally, a discussion of the considerable literature on the visual perception of words

It is this extensive review section which sets the book apart and, in a sense, exhibits its academic credentials. The result is not entirely satisfactory. One problem is that the survey has something of the texture of 'annual review' articles, dense and impressively referenced but somehow lacking in coherence. The mass of data is insufficiently organised to be readily digested by the non-psychologist or to provide new insight for the specialist. Significantly, the introductory chapters on linguistics and writing systems are much more readable than those reviewing areas of experimental psychology. There are some obscurities, such as the curious description of Spoehr and Smith's research on pages 91–92 and the seemingly contradictory claims about the effect of orthographic regularity on letter search. But on the whole the text is dry and lucid.

The treatment of underlying concepts is lengthy yet some judgements have been made about exclusion of material. Notable absences include vision research such as metacontrast, much of the post-Sperling work on letter array processing and the important work of Meyer on lexical decisions. There is also little explicit treatment of general problems of pattern recognition.

The core of the book is the section on the reading process itself This is rather more readable, not least because the view of basic questions is less obscured by an untidy forest of experimental data. This section should also prove fruitful to the non-specialist. It concludes with a review of models of skilled reading, which brings the discussion back to the preoccupations of experimental psychology. Here, in an attempt to invent some substantive issues the authors create a curious opposiion of 'information processing' and 'analysis-by-synthesis' models. This chapter also contains one of the occassional lapses into slightly partial reviewing, where (page 467) the claims of Gibson and her colleagues about an absence of acoustic or semantic effects on visual search are presented without mention of conflicting evidence.

The final section consists of short capsules for such questions as dyslexia and speed reading Of these the freshest and most absorbing is on cross-language differences in reading achievement. Here is set out the baffling lack of well established relationship between reading development and the vastly different forms of orthography. This is almost enough to terminate the cognitive psychologist's flirtation with the real world. One echoes the words of a recent article title: "Do not adjust your mind, there is a fault in reality."

Huey's classic book is a more provocative essay on reading and the recent experimental literature is more penetratingly treated elsewhere, but Gibson and Levin have produced a scholarly and comprehensive textbook. Like boxed sets of symphonies there is a simple virtue in completeness.

Leslie Henderson

Life gaining ground

Surtsey: Evolution of Life on a Volcanic Island. By Sturla Fridriksson. Pp. 198. (Butterworth: London, 1975.) £5.00.

ON November 14, 1963, a submarine volcanic eruption began in the ocean south of the Westman Islands off the coast of Iceland, which eventually resulted in the production of an island with an area of 2.5 square kilometres. The island was aptly named Surtsey, after the mythical Norse fire-giant, Surtur the Black. The geological significance of this rare event was immediately apparent, but the biological opportunities presented by the occurrence are of no lesser importance. These were fortunately recognised by men such as Sturla Fridriksson, who visited the island within six months of its formation and began the formidable task of documenting the establishment of living organisms.

The succession of life on totally bare areas of land surface has not been studied intensively, mainly because of the lack of opportunity, especially in isolated situations where invasion demands very effective dispersal on the part of the organism concerned. Students must surely be weary of the much repeated but rather fragmentary information available from Krakatoa following its eruption in 1883. But in this new book, Fridriksson has summarised the development of living communities of organisms and simple ecosystems in the 10 years since the formation of Surtsey.

The study is extensive. It documents the establishment of microbial life within months of the initial eruption, and the arrival of the first invertebrate terrestrial animals, birds and viable seeds. Complete lists of plant invaders (including bryophytes) are given, with details of when they arrived. Bird life is also covered in detail, the book largely being concerned with visiting species, but also recording with some pride the establishment of breeding pairs of fulmar and black guillemot. Arthropods are dealt with in less detail; 158 species have now been recorded, but many of these could have failed to establish themselves as permanent inhabitants.

The chapter on "Ways of Dispersal" is particularly interesting in that it deals with mechanisms by which dispersal (mainly botanical) has been effected. Some species, such as *Senecio vulgaris* and *Eriophorum scheuchzeri* have been observed during invasion by air-borne seeds. A surprising list is given of seeds found attached to the egg cases of skate and washed up on the island; many grasses arrived in that manner. Some seed eating birds (snow buntings) were found to have viable seeds in their gizzards, which could potentially provide an invasion mechanism.

Finally, the developing biota of Surtsey is compared with neighbouring islands and the mainland of Iceland, and naturally these seem to provide the major source areas for invaders.

The book is well illustrated with some spectacular colour photographs as well as a large number of line drawings and maps. It is written in a lively, informal manner, which effectively conveys Fridriksson's vigour and his enthusiastic concern to observe the ecological development of a truly pristine habitat.

P. D. Moore

Liquid column chromatography

Liquid Column Chromatography: A Survey of Modern Techniques and Applications. (Journal of Chromatography Library, Volume 3.) Edited by Zdenek Deyl, Karel Macek and Jaroslav Janak. Pp. xxi+1,175. (Elsevier Scientific: Amsterdam, London and New York, 1975.) Dfl. 290; $120 95

THIS collaborative 1,120-page volume, almost entirely the work of a group of Czech authors, is undoubtedly the most comprehensive account of the subject currently available It is divided into four main sections: theoretical aspects of liquid chromatography, techniques of liquid chromatography, practice of liquid chromatography, and applications. The first three sections are general and make up about one-third (413 pages) of the book, whereas the application of these methods to specialised classes of compounds make up the remaining two-thirds.

The general theoretical section is in some respects the least successful part of the book, as in my opinion too much attention is paid to mathematical rigour and insufficient space (only some 10 pages) to the factors which influence zone resolution in practical chromatography. The short section on the thermodynamics of partition and adsorption processes by Novak, however, is excellent. It is rather disappointing to find the old but fallacious concept of a direct relation between solute molecular weight and retention volume adopted in all the sections on gel permeation (or molecular-sieve) chromatography.

The second and third sections on the techniques and practice of liquid column chromatography are excellent The editors explain that they have decided, wisely in my view, to give equal prominence to the "classical" methods of liquid chromatography and to the newer techniques of high efficiency, high pressure chromatography, especially as the former still constitute 90% of published methods. The techniques section is essentially confined to instrumentation and materials (mobile and stationary phases) for all the fundamental subgroups of chromatography, adsorption, partition, ion-exchange and the exciting new development of affinity chromatography. The chapters (8 and 11) on instrumentation and operation of high efficiency, high pressure systems compare favourably with some of the specialised works on the subject. Chapter 9 on stationary phases and chapter 10 on mobile phases are particularly valuable, and are admirably complemented by the corresponding chapters 13 and 14 in the practice section. These sections include extensive tables of the properties of the relevant stationary and support phases, as well as those of mobile phase solvents. The criteria for choice of phase, and packing, conditioning and operation of column receive special attention. The sections throughout the book contributed by Dr O Mikes on ion-exchange chromatography and by Mrs J. Turkova on affinity chromatography must be specially commended.

The special section of the book gives detailed recommendations for the chromatographic separation of a variety of chemical compounds ranging through hydrocarbons, alcohols through carbohydrates, lipids, amino acids peptides and proteins, nucleic acids and their constituents to some specialised classes of organic compounds such as vitamins, antibodies and pesticides, to cells and subcellular particles, and finally to inorganic compounds

It is always difficult to assess the value of such large specialised sections in any book, as only a small portion of the material presented will be of any value or even interest to any particular reader, and also such material is more likely to become outdated than general sections. I believe that the best solution is a series of well-documented and indexed tables which will guide the interested reader into the literature of his own subject, without increasing the bulk (and cost) of the book with specialised material of no interest to him.

Among the best of these special chapters are 32 on amino acids by Zmrhal, Heathcote and Washington, 34 on peptides by Khluh, 35 and 36 on proteins by Prusik and Mikes, and 37 and 38 on nucleic acids and their constituents by Zadrazil. These are virtually monographs on their subjects.

In summary, this is by far the best book on liquid column chromatography to appear to date. It is excellently produced, but it is unfortunate that its price will render it inaccessible to many departmental libraries.

C. J. O. R. Morris

Dynamics of galaxies

The Formation and Dynamics of Galaxies—Proceedings of IAU Symposium No 58, Canberra, Australia, 1973. Edited by J. R. Shakeshaft. Pp. xv+441. (Reidel: Dordrecht, Holland; Boston, Massachusetts, 1974.) Cloth Dfl 135, $54.00; paper Dfl. 100, $38.50.

THE publications which arise from IAU symposia are among the most valuable books in any library used by astronomical researchers, and the present round is appearing with commendable speed, given the usual timescale for publication of proceedings volumes. Little has happened since August 1973 to reduce the value of these contributions from the leading lights in cosmology and cosmogeny who were then assembled in Canberra; on the other hand, by the very nature of the book many of the contributions have a familiar ring about them.

The editor has rearranged the record of discussion to keep comments near the papers to which they apply, and has also changed the order of presentation of the contributions to fit a more logical pattern than the exigencies of the Canberra timetable allowed. But he has not attempted to provide an overview of the state of the art, and in the field of galaxy formation more than any other in astronomy (except, perhaps, cosmology) a chapter providing such guidance would be invaluable to readers not already thoroughly familiar with the state of play.

It is no longer very unlikely that non-specialists will take an interest in the areas of study covered by this book, because the high energy astrophysical aspects, in particular, are receiving increasing attention from theoretical physicists who have a background in non-astronomical specialities. But even the congnoscenti have a real problem in distinguishing the wood of a broadly based understanding of how the matter in the Universe got into its present overall state from the many trees of particular theories which explain observations of particular objects or groups of objects without being fitted satisfactorily to the overall pattern.

If you are one of those theorists beavering away on some aspect of the overall problem, then you will find this volume useful not only in providing other models for comparison but in giving some of the observational constraints which must be met by all models. In addition, most active modellers will gain some comfort from the present state of the art; regrettable though it may be to those who treasure the idea of the Universe as a basically simple place, any overview of the work described here must surely lead to the conclusion that both the extreme cases—expansion or outbursts from nuclei, and collapse or condensation of gas clouds—play a part in the real Universe. And there are the various cases of interactions between galaxies to confuse the picture. **John Gribbin**

Organelle by organelle

The Cell in Medical Science. Vol. 1: *The Cell and Its Organelles.* Pp. xiii+366. £7.80; $20.25. Vol. 2. *Cellular Genetics, Development and Cellular Specialization.* Pp. xvii+597. £11.30; $29.25. Edited by F. Beck and J. B. Lloyd. (Academic: London and New York, January 1975.)

THESE are the first two volumes of a four volume series on *The Cell in Medical Science*, which is primarily intended to provide the basis of a cell-science course for advanced biological sciences students and for medical students specialising in cell biology during an elective period or during an intercalated year leading to an honours degree.

Volume 1 is concerned with the generalised cell unit and with the biophysical and biochemical basis of the structure and function of the chief subcellular organelles. Its eight chapters deal with the cell as a unit, membranes and transmembrane transport, the cell surface, the nucleus, cytomembranes and ribosomes, mitochondria and peroxisomes, and subcellular pathology. Though rather poorly illustrated, this volume contains a great deal of useful information and its chapters are mostly well written. That by the editors is a particularly well-balanced treatment of lysosome structure and function and lysosome pathology, pharmacology and toxicology. In my opinion, however, this volume fails to indicate (in spite of promises in the Preface) "the directions in which contemporary cell biology is moving and the methods it uses". This is because, with its traditional, anatomical, organelle-by-organelle approach, little attention is given to the current topics which excite cell biologists —for example, cell fusion and cell hybridisation, cell cooperation, nuclear transplantation, the translation of mammalian mRNA in amphibian oocytes, and the use of cell and organ culture techniques.

The first half of Volume 2 is concerned with cell genetics and development, and begins with four excellent, informative and readable chapters on biochemical genetics, general mammalian cytogenetics, human cytogenetics, and sex chromatin. A very useful chapter on the kinetics of cell production is followed by considerations of cell differentiation, growth and morphogenesis, and ageing. The remainder of Volume 2 comprises comprehensive and, on the whole, well-illustrated chapters on the main cell types and special structures of the neuromuscular system, including the neurone, the synapse, satellite cells of neurones, the skeletal muscle cell, the muscle spindle, and the smooth muscle cell. This theme of morphological adaptation for specific functional purposes will also occupy the whole of Volume 3, in which connective tissues, various endocrine cells, and absorptive and secretory cells will be considered.

In many of the chapters in these first two volumes, medical students would have appreciated more information on cellular aspects of abnormal and disease conditions, at the expense of some of the biochemical, biophysical, physiological and anatomical detail. The balance will to some extent be restored by the publication of Volume 4, which, following chapters on metabolic control mechanisms, will deal with the relationship between the cell and its environment in certain pathological states, including immunological processes, inflammation, wound healing, and carcinogenesis.

The Cell in Medical Science is an essential addition to all medical school and science libraries, where it will be valuable for teachers and research workers, as well as for senior undergraduates.

Michael Balls

Elements of pollution

Chemistry and Pollution. Edited by F. R. Benn and C. A. McAuliffe. Pp. 156. (Macmillan: London and Basingstoke, July 1975.) £8.95.

OVER the past decade there has been much talk of pollution in all its variations, unfortunately in many instances from individuals who were unaware of even the most basic knowledge of the processes involved. This book provides an introduction to the chemistry of the processes of pollution aimed at those individuals with a background of GCE "A" level chemistry; first-year university chemistry in North America. To obtain adequate in-depth coverage the editors have called on eight contributors to write the six chapters.

The stated objective of the text is to provide a factual chemical background to pollution. As might be expected, with eight authors the depth of coverage in each chapter is rather uneven and this is perhaps the most irritating aspect of the book Reference to literature and events outside the UK is rather limited and this might limit its acceptance abroad. The authors recommend the book to universities and colleges for use in environmental pollution courses at the lower undergraduate level. It is adequate for this task. There are no problems set. The book is well produced and has an adequate index.

M. A. Ward

Politics at sea

New Era of Ocean Politics. (*Studies in International Affairs*, No. 22.) By Ann L. Hollick and Robert E. Osgood. Pp.xi+131. (Johns Hopkins University Press: Baltimore and London, April 1975.) £4.40 cloth; £1.40 paper.

THESE studies are confined to US policies and interests in the oceans, the first having particular reference to the Third United Nations Conference on the Law of the Sea and the effect that the different proposals developed there will have for the US. Apart from a little more than one page entitled "International Perspective", however, no attempt has been made to look at the policies from a global standpoint. Having said this, however, it should be remarked that the studies are a very useful contribution to the literature on the Law of the Sea and every participant in the UN Conference should own a copy, or at least have access to one, so as to gain an insight into both the US position in their particular fields and the reasoning behind US statements and activities.

Ann Hollick's study is of greater interest to the marine scientist even though, as is inevitable in a field such as this, it was partially obsolete before it was published, dating from the end of the third (Caracas) session of the Conference in mid-1974; even the outcome of that session is only lightly touched upon. Much, too, has since happened at the recent, fourth session in Geneva.

Consideration of the US position on Marine Pollution and Scientific Research has been confined to some six pages which discuss, first, the reason for including marine pollution within the competence of the conference (this happened because of Canadian insistence after the Manhattan voyage through the North-West Passage in the summer of 1969) and second, how late in the proceedings (summer 1973) the US formulated a policy of Scientific Research; before that, US policy had been almost entirely dominated by petroleum-defence interests. Nonetheless, the US was one of the first countries seriously to consider marine scientific interests at all.

The second study, by Robert Osgood, is an interesting account of US security (that is, military) interests in the oceans, and the surrounding legal implications. This section is of lesser concern to the marine scientist though it does show why US policy has developed as it has, sometimes to the detriment of scientific interests.

The book is well produced and readable in spite of its specialist interest. This is partly a result of a number of fascinating insights into the workings of US government (no doubt others operate in similar fashion but one seldom gets the opportunity to read about them).

Desmond P. D. Scott

obituary

Gordon Hamilton Fairley, a leading authority in the drug treatment of cancer, was killed by a bomb explosion in London on October 23. He was 45.

PROFESSOR Hamilton Fairley began his clinical studies at St Bartholomew's Hospital, London, qualifying in 1954. From 1958–61 he held the Leverhulme Research Scholarship of the Royal College of Physicians During that time he began his research into the immune mechanisms of blood diseases In 1965 he was appointed to the consultant staff at St Bartholomew's, where in 1970 he became director of the Imperial Cancer Research Fund Medical Oncology Unit. He was appointed professor at the unit two years later. Much of his time was spent in work on tumour immunology and in improving drug combinations used in the treatment of leukaemias and related disorders, such as Hodgkin's disease.

As reality replaces grief, the magnitude of the loss of Gordon Hamilton Fairley becomes increasingly clear, and facets of his work, taken for granted, suddenly become irreplaceable. It seems difficult to realise that only a decade has elapsed since Gordon directed his attentions to a new approach to cancer research. Following his sound clinical training in oncology, gained under Sir Ronald Bodley Scott, he chose to develop a close liaison with pure scientists to develop a rationale for future clinical studies. During this period he became a close friend of Peter Alexander, a scientist without clinical training, and between them they taught each other much of their respective disciplines. Basic scientific observations were rapidly reported, particularly in tumour immunology, and tested in clinical programmes. One of his major contributions was to lay down rigid principles concerning these clinical programmes, particularly the concentration of efforts into only a few diseases at any one time, and the use of carefully controlled randomised clinical trials He had the remarkable knack of keeping the programmes relevant, while keeping an open mind for scientific developments that were important. Using these principles his contribution to the fields of leukaemias and lymphomas was considerable He had the ability to be effective without making enemies, because of a personality and charm with a common purpose, without intertive smile could shorten committee meetings by hours. It meant that a unit was created which was bound together with a common purpose, without internal squabbles; and his generosity to those who worked for him instilled into them a quality they passed on to others He was responsible for training half the medical oncologists at present practising in Britain, and his monument will be the living contribution yet to be made by these people. All these qualities made Gordon an obvious choice to steer the direction in which medical oncology takes in Britain. Because he was so young, in this he is irreplaceable. With his death we lose the founder of British medical oncology, but more tragically to many of us we lose a dear friend.

Ray Powles

B. A. Southgate, CBE, a leading British researcher in problems of water pollution, has died at the age of 71.

Shortly after leaving Cambridge, he joined the recently formed Water Pollution Research Organisation (part of the former Department of Scientific and Industrial Research) as a member —and ultimately leader—of a team investigating the effects of pollution on the Tees estuary Subsequently, many other such surveys were carried out under his direction, the most important of which was that concerned with the Thames estuary. A predictive mathematical model was developed to establish the best way of improving conditions in the estuary. This research was to form the basis of a new kind of approach to river management. At the outbreak of World War II, Dr Southgate set up the first permanent Water Pollution Research Laboratory (WPRL) in the UK at Watford and was appointed director in 1943. In 1948 he published *Treatment and Disposal of Industrial Wastewaters,* for many years one of the standard reference works. He was awarded a CBE in 1954, the year in which his laboratory moved to Stevenage. After retiring from WPRL in 1966 he continued to act as a consultant to relevant organisations in the UK and in the United Nations.

announcements

Appointments

D. A. Hamburg has been appointed president of the Institute of Medicine, Washington, D.C.

The new pro-vice-chancellor of the City University, London is to be **J. C. Levy.**

E. C. Melby, Jr., has been appointed chairman of the Institute of Laboratory Animal Resources of the National Research Council in Washington, D.C.

Award

The Gairdner Foundation of Ontario has presented one of its 1975 awards to **H. E. Huxley** in recognition of his outstanding contribution to our understanding of the molecular basis of muscle contraction.

Miscellaneous

A workshop on **biological surveys of estuaries** is to be held at UWIST, Cardiff, UK from January 5–9, 1976 A limited number of workshop places are available for river biologists For further details apply to:—Technical Presentation Manager, Water Research Centre, Stevenage Laboratory, Elder Way, Stevenage, Herts, SG1 1TH, UK.

Scientific visits to the Soviet Union, Czechoslovakia, Hungary, Romania and Bulgaria. Cultural agreements with the above countries provide for short visits (two weeks) and medium term research visits (two to six months) for British scientists. Scholarships are also available for periods of up to one year. For full details apply to:—Higher Education and Science Department, The British Council, 10 Spring Gardens, London SW1A 2BN.

Reports and publications

Other countries

Annals of the South African Museum. Vol 67, Part 5· The Amphipoda of Southern Africa. Part 5. The Gammaridea and Caprellidea of the Cape Province West of Cape Agulhas. By C. L. Griffiths. Pp 91–181. R.5 60 Vol 69, Part 2. *Thermally* Anomalous Late Pleistocene Molluscs from the South-Western Cape Province, South Africa. By Anthony J. Tankard Pp. 17–45. R.2.90. (Cape Town South Africa Museum, 1975) [199

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Person to Person

London-Washington. Family house (three young children) sought in Bethesda (NIH)–Washington area for 3 months in exchange for three-bedroom North London house (Dr David Brown, Department of Pharmacology, The School of Pharmacy, London University, 29/39 Brunswick Square, London WC1N 1AX, UK; (01-837 7651 ext. 55).

London—South Carolina. Two- or three-bedroom house wanted in London area for professor on sabbatical from May until December 1976 in exchange for four-bedroom house near South Carolina University. Car may be included (Professor J. L. Safko, Department of Physics, University of South Carolina, Columbia, S.C. 29208, USA).

Eggshell thinning in Falco. Eggshell thinning of several species of raptoral birds has been demonstrated in the British Isles and North America. Drs David Peakall and Lloyd Kiff are attempting to document this change on a global scale. They would be interested to know the whereabouts of any post-1945 eggs of *Falco peregrinus*, *F. biarmicus*, *F. jugger* and *F. cherrug*. The only exception is *F. peregrinus* collected in the British Isles. The measurements required—length, breadth and weight—can be obtained easily without damage to the specimen. They would be grateful if anyone knowing the location of these eggs would write to them (David B. Peakall, Canadian Wildlife Service, Department of the Environment, Ottawa, Ontario K1A OH3, Canada, or Lloyd F. Kiff, Western Foundation of Vertebrate Zoology, 1100 Glendon Avenue, Los Angeles, California 90024, USA).

Human zygotes. Information is required on a hypothesis on the sex of human zygotes. For this purpose will anyone with data on the sex of any monoamniotic twin pairs please communicate with Dr W. James, Galton Laboratory, University College, London, UK

There will be no charge for this service. Send items (not more than *60 words*) to Holly Connell at the London office. The section will include exchanges of accommodation, personal *announcements* and scientific queries We reserve the right to decline material submitted No commercial transactions.

nature

November 20, 1975

Questioning the quinquennium

THE practice whereby British universities are financed on a quinquennial basis is the root of many of their present problems. In fact, the question now seems to be not so much if, but when, that system will be done away with or modified out of all recognition. The problem is that the arrangements for correcting (for higher-than-expected inflation rates) a particular year's expenditure—planned at the beginning of the five-year period—is cumbersome and drawn out. One of the universities' most immediate complaints, for example, is that their grant for the next academic year, 1976–77, is not yet fixed even though they are already admitting students for courses starting next October and have therefore had to make some fairly detailed plans. One suspects that even the government's much-touted but tentative ideas for introducing cash limits on public expenditure, with no *post facto* corrections for 'extra' inflation, would be welcome, relatively speaking, because at least the universities would then know where they stood.

An added complication, of course, is that the whole business of salaries for university academics is in disarray. Figures put out recently by the Department of Education and Science show how they have altered, at 1970 prices, over the past five years. The lecturer scale has just about kept up with inflation, and the recent Board of Arbitration settlement means that lecturers' salaries as of October 1974 (which is as far as the negotiations have got!) are some 20% ahead of 1970 in real terms. But senior lecturers and professors, who can hardly be said to earn astronomical salaries, had until the recent settlement fallen seriously behind.

The average professorial salary in 1970 was £5,610, whereas by 1974 it had shrunk a startling 12% to £4,920 at 1970 prices. The level for that year was effectively increased to £5,905 (1970 prices) by the recent settlement. This only put professors about 5% ahead in real terms, and that has been more than eroded in the past year.

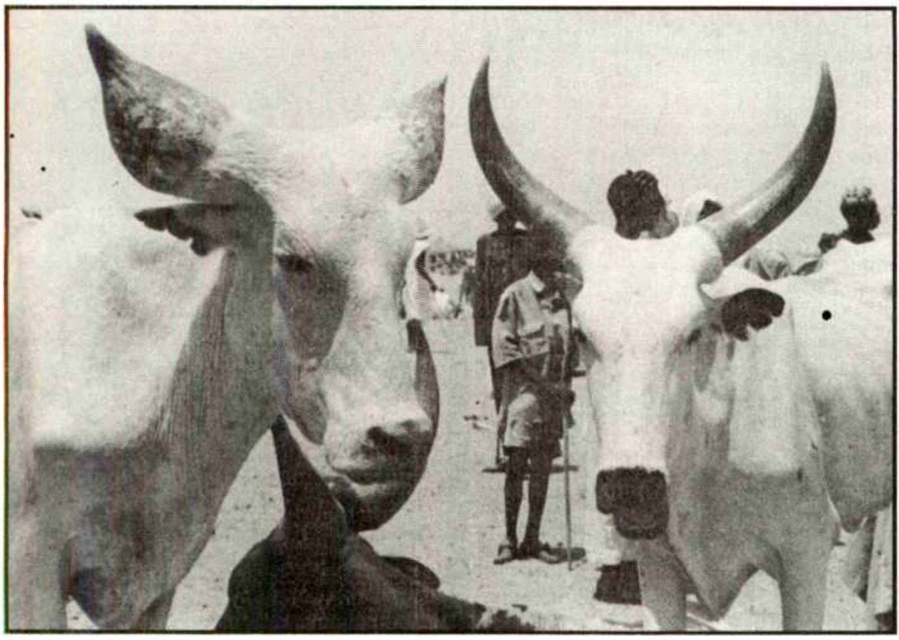
Planning anything is difficult for everyone, including the Department of Education and Science. Bearing in mind that academic salaries account for some 50% of university non-capital expenditure, planning is even more difficult when it comes to universities. But is it an excuse for inaction?

Where have all the teachers gone?

THE shortage of science teachers in British schools seems likely to intensify, with a consequent loss of potential scientists and engineers. This gloomy prognostication emerged recently when educationalists from varied backgrounds gathered at the Royal Society in London to consider problems in the teaching of science and mathematics. The meeting also demonstrated the apparent gap between the aims of many school teachers and the knowledge and attitudes required of first-year students at colleges and universities.

The shortage of scientists and mathematicians in schools, which exists in spite of the overall national sufficiency of teachers, will be exacerbated by the cuts in the numbers and sizes of teacher training colleges, recently necessitated by instructions from the Department of Education and Science. Where small departments within colleges have to be closed down they may well include those concerned with science, so that even fewer primary and secondary school teachers will be available with the backgrond necessary to teach science effectively. Another disturbing factor will be the loss of flexibility in training colleges which are amalgamated with polytechnics and adopt courses leading to the BEd degree of the Council for National Academic Awards. These courses will require students to choose their main subject from the outset, rather than specialising only after a broad, basic course including mathematics and science. During a wide ranging basic course students can be persuaded, by imaginative teaching methods, that science is not necessarily boring, rigid and too difficult, as they may have thought at school. In these circumstances students might opt to specialise in science or mathematics after the first few terms, but when the choice has to be made at the beginning of the first year the chances are that prejudices acquired at school will have an undue influence.

School teachers who are trying hard to arouse enthusiasm for science and mathematics among their pupils, through the use of new methods and curricula, are apparently coming into conflict to some extent with the needs of colleges and universities. The contributions of academics to the meeting at the Royal Society suggested that students who have learned about theories and concepts at an early age do not all have sufficient factual knowledge when they embark on higher scientific education. It is more important, the academics say, to understand the meaning of *p*H than to be familiar with molecular orbital theory. But the school teachers counter that they have to interest their pupils in science, and this is not necessarily done by constant learning of facts. The teachers ask whether they are supposed to be preparing pupils to enter higher education or to become effective citizens. This is a question that will have to be considered carefully in future deliberations about the structure and content of British education.



Kuri cattle at Grédaya market, Chad

Conserving genetic resources

Many breeds of livestock and innumerable varieties of crops which have arisen in the course of man's agricultural history are now in grave danger of being supplanted by the comparatively fewer highly-bred animals and plants of modern agriculture. A priceless and irreplaceable reservoir of genetic raw material is threatened. Peter Collins looks at efforts now underway to preserve some of the rapidly vanishing breeds of livestock. Next week Eleanor Lawrence reviews international efforts to conserve crop genetic resources.

CONCERN for the fate of traditional breeds of livestock is an aspect of conservation that has as yet received little attention at the international level. It is true that cerain breeds, especially of cattle, that would otherwise have disappeared have been preserved by societies set up for this specific purpose. But most breed societies are at least as much concerned with the quality and performance characteristics of the breeds they represent as with conserving them as sources of genetic material. This is by contrast with the tremendous interest now apparent, and actively fostered over many years by the Food and Agriculture Organisation (FAO), in maintaining genetic stocks of crop plants.

Until fairly recently the specialised breed society was largely a British institution. Although this movement is now spreading, breed societies are still confined principally to the agriculturally more advanced countries, but even they have not prevented the disappearance of a number of ancient and potentially valuable breeds. Nowhere outside the UK is there anything approaching the Rare Breeds Survival Trust, a body specifically concerned with the conservation of British breeds of livestock that would otherwise become extinct.

Pure stocks of major commercial breeds have, of course, been maintained, especially where their performance is particularly in line with present thinking in the agro-economic field. But the number of such breeds has been declining for many years, and this process is accelerating rapidly. Thus, a recent FAO publication (*Report of a Pilot Study by FAO and UNEP on Conservation of Animal Genetic Resources,* Rome, March 1975) identifies 118 breeds of cattle in Europe and the Mediterranean Basin as being "in a relic state, in danger now, or in danger in the future." It records only 30 native breeds from the same group of countries as "not in danger".

Among those already threatened with extinction are such familiar breeds as the Irish Kerry ("already in a relic state"), and the Red Poll and Beef and Dairy Shorthorns in the UK; even Scotland's Ayrshire is in danger of disappearing. The decline is particularly marked in the dairy breeds, as it is throughout Europe, on account of the tremendous rise in popularity of various types of Friesian. There has always been a certain amount of deliberate cross breeding in the livestock industry, relying on the maintenance of purebred stocks of the selected parent breeds. This aspect of conservation has scarcely been recognised, mainly because the objective has been increased performance and production of more and better meat and milk, and occasionally, disease resistance.

But it is this point of view that has, for very good reasons, principally influenced the FAO's approach to the problems of increasing livestock production in the developing countries. Thus, the FAO has frequently advocated the use of local breeds in these countries for crossing with introduced animals, but has not been greatly interested in the conservation of other, less immediately useful indigenous breeds. Now, there is increasing concern that the success of such introductions is beginning to erode the irreplaceable genetic resource that exists in traditional native African and Asian breeds. Many of these, it is feared, may disappear before there has been a chance to evaluate their performance under improved conditions, or for cross breeding with other local or introduced breeds.

With the sudden surge of interest in conservation, and more particularly, with the creation of the United Nations Environment Programme (UNEP), this situation has begun to change. UNEP has a specific objective in this field, based on a resolution of the Stockholm Environment Conference of 1972. This is "to initiate the preparation of a comprehensive catalogue of threatened species and varieties of crop plants, fish, domestic animals and micro-organisms, and to cooperate with the FAO in its programme for genetic resource conservation . . ."

Earlier this year the FAO and UNEP published a report of their first joint project. The project—in effect, the first international step towards the conservation of genetic resources of domestic animals—has two objectives. First, "to prepare a preliminary list of breeds of farm animals which are in danger of extinction together with an account of any measures which have been recommended or taken to prevent this extinction"; and, second, an examination of the problems involved in the conservation of a single breed in a developing country.

A breed of humpless cattle known as the Kuri was chosen for the second part of the project. The Kuri, whose habitat is the islands and shores of Lake Chad, is unique in the possession of inflated, spongy horns, apparently (though exactly how is not known) an adaptation to its habits and habitat. A fine swimmer, the Kuri actually feeds in Lake Chad, eating the coarse forage of the shallow shores, and swims to and fro to reach new pastures. The concern about the survival of the Kuri, however, is based on a series of circumstances affecting, or liable to affect, many other breeds in developing countries.

Since 1960, a rise in the water level of Lake Chad seems seriously to have reduced the available grazing area. Then, because of the demand for Kuri stock, which are described as moderate milkers and excellent beef animals, local farmers could obtain two zebu cattle for one Kuri. This cannot be avoided where numbers of cattle rather than quality is still important to local tribes, but the consequence is dilution of the Kuri herds with zebu blood. In addition, the Sahelian drought of recent years has led to pressure on the Kuri grazing areas by zebu herds from the Sahel.

The original project for the conservation of the Kuri was proposed in 1972 by the Lake Chad Basin Commission. The aim is to establish several herds of pure-bred Kuri, selected with an emphasis on the animals' economic qualities, but including one small herd specifically intended to preserve the unique, inflated, buoy-shaped horns which have attracted many travellers in the Lake Chad region—and which could make these Kuri a tourist attraction in themselves. In its latest form, with UNEP–FAO assistance, this project can be seen as a valuable exercise in the deliberate conservation of a threatened breed.

While most attention has so far been directed towards cattle, the situation is little different where other farm livestock are concerned. Many breeds of sheep, pigs, horses and even goats are in danger of becoming extinct, mainly for the same reasons: changes in the pattern of farming, improvement of land, and the constant demand for higher production based on the use of feeds not available in the 'natural' environment. Other domesticated animals, such as the camel and its South American relatives, have scarcely been considered from the conservation point of view; a minor species such as the donkey could well disappear simply because no one has thought of doing anything to prevent this happening.

In general, there are three aspects of this conservation problem: cultural, scientific and economic. The traditional breeds of livestock are, first of all, as much a part of our cultural heritage as are ancient buildings and works of art. From the scientific point of view, certain breeds have colour genes. The larger species, too, are useful as controllable sources of milk, blood and various tissues of particular genetic interest.

But it is the practical economic argument that is the most important. The classic example of this is probably the "discovery" by American poultry breeders of the traditional Cornish breed, reduced in its home country to a purely fancy bird. Crossed with other breeds, it made possible the rapid growth on which the modern broiler industry depends.

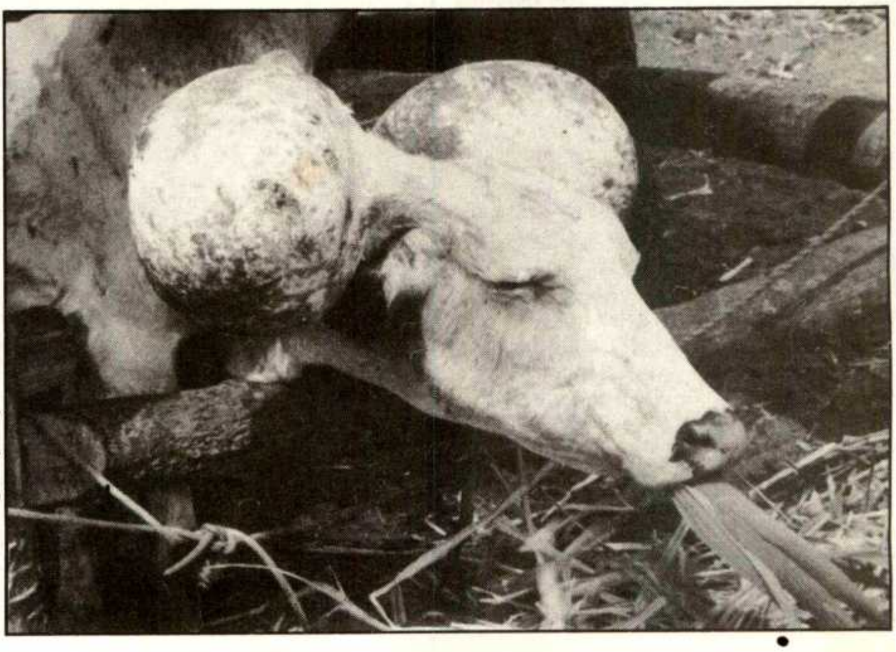
Similarly, remote breeds of sheep, for example from Greece and Finland, were unknown beyond their own homelands until, because they were highly prolific, they were seen to be suited to modern husbandry techniques. There are several very prolific tropical breeds of sheep, too, in the Caribbean, in Latin America, West Africa and South-east Asia, that could also be useful.

An even more important example is probably that of the 'trypanotolerant' indigenous cattle of various West African countries. If these could be successfully crossed, or developed under improved conditions to provide a more economic yield of meat and milk, it might be possible to avoid the enormous cost of controlling tsetse fly, the vector of trypanosomiasis, which denies thousands of square miles of Africa to the livestock industry.

One apparent problem, however, is the lack of knowledge of the characteristics of most of the indigenous breeds of the developing countries. A first step, which must come before a conservation programme can be set up for any breed, is obviously its evaluation in terms of production, disease resistance, and potential when crossed with other, better known breeds. This means a long and enormously costly series of projects.

Moreover, even where there have been apparently successful cross-breeding programmes, little is sometimes known about what is happening from the genetic point of view. Thus, it was long considered that the "hybrid vigour" (heterosis) apparent when certain exotic, highly developed breeds have been crossed with local stock, was due to the introduced, often larger animal. Now it seems that this is not necessarily the case, and it is essential that sufficient pure-bred herds of the local breed be maintained if such cross breeding is to be continually successful. An example is quoted of the local Criollo cattle in South America, which have been successfully crossed with Brahman (zebu) bulls from the United States. Now the Criollo themselves are in danger of dying out, and the genetic resource they represent could be lost.

Conservation of livestock breeds for economic or cross-breeding purposes demands the maintenance of considerable herds of pure-bred stock, which many of the poorer developing countries may not be able to afford. This therefore is a case for international action. Where a breed is being maintained for scientific purposes, smaller herds, such as might be kept in rare animal parks or large zoos such as Whipsnade, will suffice. Where the interest is solely cultural or historic, such as in the case of the UK's White Park cattle at Cadzow and Chillingham, even fewer beasts need be kept and the problem of cost may be less important than that of management. Certainly, some of the hundreds of breeds of livestock now kept on the world's farmlands will disappear. But with the new approaches to agriculture, and the call to combine higher production with better use of animal and plant resources, everything must be done to ensure that as little as possible of the genetic heritage available in these breeds is lost. ☐



Kuri cow showing buoy-shaped horns

Relative deprivation of Civil Service scientists

SENSIBLE negotiators will use the breathing space provided by the British Government's £6-a-week pay policy to seek solutions to their long standing industrial relations problems. One such problem is that of the pay and careers of Civil Service scientists, including those employed in research councils, the UK Atomic Energy Authority and other closely related areas of employment.

Civil Service scientists are highly discontented. They have become more frustrated since the hopes raised by the Fulton Report on the Civil Service that equal opportunities would be given to specialist staffs have been seen to come to naught. It is difficult to recall a time in the past 30 years when they have so resented the comparative injustices which they suffer.

Why should this be so? The best point at which to begin is with the recruitment of staff. The Civil Service recruits graduates with a First or Upper Second Class Honours degree—and, perhaps, a second degree—separately to the two main groups, Administration and Science. Although in both cases recruits will meet the academic requirements, there is very little doubt that in practice the scientist will be the more highly qualified.

After a year or two of service the administrator, at every grade level he reaches below £12,000, at which point there is unified grading, will be paid more than his science counterpart. The difference is of the order of 3.5–5.5% at comparable salary maxima. The relationship is similar in respect of A-level entrants to each of the groups.

But this is far from the whole story. The salary differences—themselves impossible to justify and a constant source of irritation—are aggravated further by the fact that career progression in administration is greatly superior to that in science. Consider the example of the high quality graduate, with a First or Upper Second Class Honours degree. The scientist will get to Higher Scientific Officer in his late 20s (only 2.3% are under age 25), to Senior Scientific Officer in his 30s (8.6% under age 30) and to Principal Scientific Officer (£7,205) in his late 30s (0.7% under age 30). A handful will get there more quickly.

Compare this with the high quality graduate administrator. He will become a Principal (£7,450) between the ages of 28 and 32. It is hardly surprising that he gets there more quickly than most scientists. There is an additional grade (SSO) in the science structure. The administrator will, as a matter of virtual certainty, progress to Assistant Secretary (£11,000) in his early 40s.

Thus the high quality graduate administrator can normally expect to progress to a salary level some £4,000 above that of the scientist. The ratios of promotions above Assistant Secretary (£11,000) for the administrator are broadly similar to those for the Principal Scientific Officer (£7,205) on the science side but, of course, at each stage at one step higher.

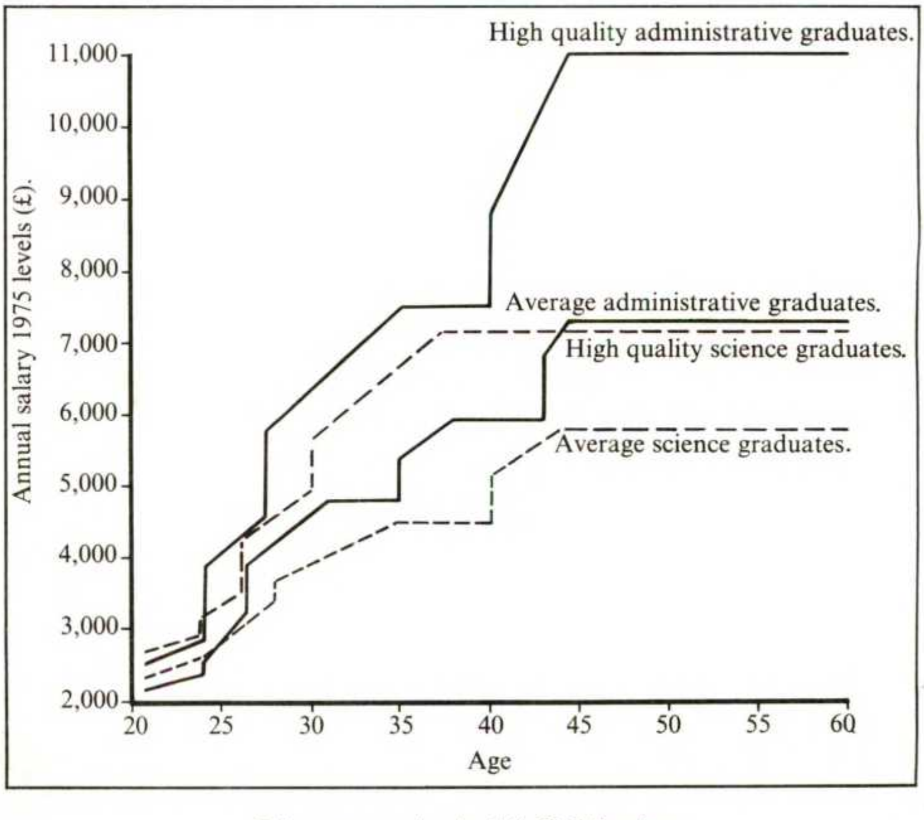
The career earnings of the administrator are thus very much more than those of the scientist, as the pattern illustrates. For the situation in which neither the administrator nor the scientist progresses further than indicated—and the chances of doing so are marginally greater for the administrator—a comparison of their career earnings between the ages of 21 and 60 shows that the best administrator will receive 34% more than the scientist. A comparison for average staff shows the administrator with a 24% advantage.

In 1974 there were 1,100 vacancies for science graduates in the Civil Service proper. Posts were offered to 1,415 applicants, but only 661 actually took them up. It is very unlikely that lack of job interest or of good facilities for research were the explanation for this. There can be no doubt that pay and career prospects played a major part in the decision making. In fact, scientists would all be better off financially if they entered the Civil Service as administrators.

Yet, odd as it may seem, very real obstacles are placed in the way of scientists who wish to transfer, later in life, to administration, in spite of the good intentions of the Civil Service Department. In this the Civil Service differs from industry which draws heavily on scientists to fill posts at large.

Civil Service scientists are not obsessed with jealousy of administrators. They do not at all begrudge administrators what they have. But scientists do not see themselves as in any way inferior. They consider that the intellectual contribution of the scientist is worth at least as much by way of pay and career prospects as the contribution of the administrator. They ask no more than that there should be equality of opportunity for all Service recruits of equal ability. Could not all of us agree about that as a reasonable objective? As things are at present scientists are not even in the same race as administrators.

Cyril Cooper



Salary expectation in UK Civil Service

international news

Busting the FDA

by Colin Norman, Washington

A MAJOR debate is shaping up in the United States over the manner in which new drugs are developed, tested and marketed. Although the issues are hardly novel and the arguments are likely to be shrill, the debate could eventually result in the first sweeping changes in food and drug laws since drug regulations were tightened in 1962 following the thalidomide disaster in Europe.

The focus of the debate will be a bill, drafted by Senator Edward M. Kennedy, which seeks to dismantle the Food and Drug Administration (FDA) and to strengthen and streamline the process by which new drugs are tested and approved. Although the bill has not yet been formally introduced into the Senate, it is already generating considerable discussion.

The impetus behind the bill comes from a series of highly publicised hearings, conducted over the past couple of years by Kennedy's health subcommittee and by a subcommittee on Monopoly headed by Senator Gaylord Nelson, during which the FDA has been flayed from all sides.

On the one hand, a number of scandalous practices in the sale, promotion and testing of drugs have been unearthed, and the FDA has been accused of sloppy, ineffective and even biased regulation of the drug industry. In short, it has been alleged that the American public is being bilked and its health endangered by unsafe and ineffective drugs which have been allowed on the market with inadequate testing and review. The drug industry, which was once widely admired and respected for its contributions to public health has—in some cases deservedly—become an increasingly plump target for criticism.

But, on the other hand, a phalanx of economists, pharmacologists and physicians has argued that the American public is being short-changed for an entirely different reason : the drug laws are now so strict, and the approval process for new drugs is so tortuous, that innovation is being stifled and patients are being denied valuable drugs which are available in other countries, especially Europe.

Whatever the validity of the various charges, it is clear that some changes in drug laws will have to be made. As Mr Kennedy, whose chairmanship of the health subcommittee gives him a pivotal role in such matters, put it during a recent symposium at Tulane University, "there are fundamental defects in our nation's regulatory procedures for prescription drugs, and these defects can no longer be ignored".

Kennedy's bill attempts to bring about a variety of improvements in the situation by breaking up the FDA and replacing it with two separate agencies —a Drug and Devices Administration and a Food and Cosmetics Administration. It also seeks to strengthen the scientific competence of the staff which reviews applications to test and market new drugs; improve and, in some cases, shorten the review process; and establish a system for monitoring clinical experience with new drugs.

The 1962, post-thalidomide amendments to the food and drug laws established a testing procedure which must be followed before any new drug can be marketed in the United States. The goal of the process, which is monitored and licensed by the FDA, is twofold: to determine that drugs meet safety requirements, and to demonstrate that they are effective. The tests, in short, proceed from animal experiments, through limited toxicological tests on humans—usually prisoners—to broader clinical trials to determine effectiveness. The procedure is rigorous and expensive.

Criticis of the system who claim that the regulatory filter allows dangerous and ineffective drugs to slip through generally tend to blame the administration of the laws rather than the laws themselves for endangering the health and wallets of the American public. Their central allegation is that the FDA is so heavily dominated by industry pressure that its decisions are frequently biased in the industry's favour. As evidence, they cite the 'revolving door' syndrome in which top FDA personnel are often drawn from the drug industry and usually return to it. And scarcely a month goes by without a Congressional committee hearing evidence that some drug or another is unsafe or useless.

Moreover, the air of public mistrust which hangs over the drug industry doesn't help the industry. The mistrust is understandable, in view of a string of allegations which have been voiced at the Senate hearings and in various reports and studies.

To take just a couple. Last year, four former drug salesmen told Kennedy's subcommittee in considerable detail of the scandalous gimmicks and hard selling tactics which they used in peddling their wares to prescribing physicians. The tactics ranged from perusing the files of pharmacies to see which physicians were prescribing their company's drugs, to the industry's practice of sending 2,000 million free samples through the mail to physicians each year. And recently, wide publicity has been given to a study, supported by Consumers' Union, which has unearthed a number of instances of drugs which are considered dangerous or ineffective in the United States, being marketed by multinational companies, with limited controls, in Latin American and Carribbean Countries.

But the critics' most widely publicised piece of evidence of alleged industry favouritism within the FDA cropped up in August last year, when 10 present and former employees of the agency testified to Kennedy's subcommittee that they had been harrassed or transferred, or that their decisions had been countermanded, when they took action against some drugs. Those allegations immediately brought promises from the Department of Health, Education and Welfare, FDA's parent department, that a thorough investigation of the matter would be conducted. A seven-member panel was eventually established, under the chairmanship of Dr Thomas Chalmers, Dean of the Mount Sinai School of Medicine, to look into the charges. And the FDA also began its own investigation.

The FDA exonerated itself in a massive, 906-page report on the charges last week, but its conclusions were, understandably, not immediately accepted by the critics. The Chalmers committee is expected to complete its investigation early next year, and its report will clearly play a substantial role in the debate on Kennedy's bill.

As for the allegations that the drug laws are so strict that they are stunting the development of new drugs and depriving patients of valuable products, those charges were first seriously raised in 1972 by economist Sam Peltzman of the University of Chicago, and they have since been extended by William Wardell and Louis Lasagna, both of whom are pharmacologists at the University of Rochester. Their studies have recently been published by the American Enterprise Institute, a conservative Washington 'think tank'.

Peltzman examined the rate of introduction of new drugs in the United States before and after the 1962 amendments to the drug laws, and compared his findings with experience overseas. He found that innovation was markedly reduced by the amendments, and he concluded that the net cost to consumers of the delayed introduction or non-availability of new drugs amounts to about $250–$350 million a year, over and above any benefits derived from the regulations.

Wardell and Lasagna compared the availability of drugs in the United States with the situation in Britain, where efficacy requirements are not as rigorously enforced, and they concluded that valuable new drugs are marketed more quickly in Britain, and that some drugs widely used in Britain are still not available in the United States.

The FDA's most vigorous and elegant rebuttal of the Peltzman–Wardell–Lasagna thesis was made in 1973 by Dr Henry Simmons, then Director of FDA's Bureau of Drugs, in testimony before Senator Nelson's subcommittee. Citing studies conducted for the FDA by the National Academy of Sciences, Simmons pointed out that of 4,300 drugs marketed before the 1962 amendments came into effect, only 40% showed substantial evidence of effectiveness. He also accused doctors of over-prescribing drugs, and said that adverse reaction to drugs has now developed into a major health problem. It was precisely because of such factors that the 1962 amendments were passed. Simmons also suggested that the FDA has serious reservations about the safety of some drugs that are available abroad, and he cited the lack of any thalidomide-like tragedy in the United States as evidence of the effectiveness of the drug laws.

Senator Edward Kennedy

Kennedy seems to have come to the conclusion that there is more than a grain of truth in the criticisms of both sides. In his speech at Tulane University, he acknowledged that the FDA has some serious problems, but he suggested that they stem from the agency's "incredible range of unrelated responsibilities, and its insufficient scientific expertise". That explains why his bill would cleave the agency into two separate units, each of which would have more manageable responsibilities.

As for the scientific quality of FDA's staff, a host of investigations of the agency carried out over the past decade or so have almost unanimously suggested that the quality needs to be improved. That conclusion is hardly surprising. Because the Bureau of Drugs is spread over six different buildings in Washington alone, there is little opportunity for FDA officials to do research of their own and, as FDA Commissioner Alexander Schmidt recently put it, "opportunities for professional growth are meagre". Kennedy's bill would therefore beef up the quality of the scientific staff by reserving a number of positions for academic pharmacologists to serve in the agency for two or three years, with ample research time and equipment to carry out their own research.

There is, however, at least one aspect of drug regulation in the United States on which most people agree: once a drug is approved and marketed, there is very little follow-up to monitor for unexpected side-effects, or for possible new applications. As Kennedy suggested last week, "the absurdity of the system is staggering. We clear a drug for a particular use. We are meticulous about requiring proof of effectiveness for that use. We spend millions of dollars and years of time developing that proof. But once a drug is finally approved, it is turned loose, free from all controls and follow-up".

FDA does run a system for receiving reports of adverse drug reactions, but a recent report by the Congressional General Accounting Office found the system to be hopelessly inefficient, and even Mr Schmidt acknowledges that "the greatest single weakness of our present regulatory system is the abysmal reporting by almost all professionals of their drug experience—good, bad or indifferent".

Kennedy's bill seeks to take care of those complaints by changing the regulatory process in such a way that some drugs could be more closely monitored and others will be brought more quickly into use. He has proposed that before some drugs are given full commercial approval, they should be placed on a limited distribution to a few hospitals, or to a particular region of the country, and their use should be closely watched. The proposal would allow drugs which may have potential problems to be closely monitored, and it would also enable products which seem especially promising, and whose safety has been reliably determined, to be brought more quickly into use—limited distribution could be substituted for some of the time-consuming and costly testing for effectiveness.

Mr Schmidt said, also in a speech at Tulane University earlier this month, that the proposal "might permit earlier appearance in the United States of many drugs, in return for a longer investigational phase controlled by FDA. This seems to me a reasonable trade-off". And Dr Wardell said in a telephone interview last week that he believes the proposal has some merit, provided that limited distribution and monitoring of new drugs is not simply added to the already lengthy review process.

As for the suggestion that FDA should be split in two, Mr Schmidt declared himself opposed. The regulation of food and cosmetics, he said, is similar to the regulation of drugs and medical devices, and the two functions can effectively share a central legal and enforcement division. Nevertheless, he acknowledged that "when Senator Kennedy suggests changes in FDA's structure, we are inclined to pay attention".

Kennedy has promised to send his bill to interested parties as soon as it is formally introduced in the Senate, and he will conduct hearings on it next spring. With Congress and the White House both battened down for the elections by then, it is unlikely that the measure will be passed before the end of the year. But the bill will very probably form the basis for legislation in the not too distant future.

● To help pave the way for the science policy office which will soon be re-established in the White House, President Ford has established two top-level committees of scientists to advise the Administration on a number of important science policy issues. One, chaired by Dr Simon Ramo, will "examine issues and opportunities involving the fostering economic strength and in improved utilisation of technology in assuring that economic goals are achieved along with environmental goals", according to a White House announcement. The other, chaired by Dr William O. Baker, will "consider developments that may take place in science and engineering in the decade ahead and examine the national policy implications of these developments". Members of both committees are distinguished and well-versed in Washington science advisory activities. ☐

Soviet scene

from Vera Rich, London

THE former President of the Soviet Academy of Sciences, Mstislav V. Keldysh, who retired from his post earlier this year on the grounds of ill-health, seems to have made a recovery, judging from recent articles in the Soviet press. On November 8, the day following the announcement of this year's State Prizes, it was he, and not his successor Academician Vladimir A. Kotel'nikov, who gave the traditional appreciation of the achievements being honoured. A few days later, on November 16, he was again featured in *Pravda,* in an extensive interview on the success of the recent Venera 9 and 10 probes.

Neither of the articles is in any way out of context. Keldysh is still Chairman of the Lenin and State Prize Committee for Science and Technology, and was closely involved with the Soviet space programme in its early days. Nevertheless, those who suspected some connection between Keldysh's retirement and the postponement of the Academy's Jubilee Celebrations from May 1974 to October 1975 will doubtless find something more than fortuitous in his sudden reappearance in print so soon after the conclusion of the celebrations.

One interesting feature of this year's State Prize lists is the emphasis given not only to basic research and applied technology, but also to the production of text books. Although in the past Lenin or State Prizes have been awarded to authors in connection with a specific scientific monograph, this year three works intended for students' use have been specifically honoured. They represent a broad spectrum: ordinary differential equations, general surgery, and radio receivers.

The Soviet academic system makes a sharp division between pedagogic and research activities—the universities confine themselves entirely to teaching, while original research is carried out only in special institutes. Although a research scientist will frequently hold more than one post, combining research at an appropriate institute with university lectures, his main chance of official recognition in the State and Lenin Prize lists has until now been through his research.

This new emphasis on the importance of text books is an interesting sidelight on the growing concern of Soviet educationalists who want works of "a high scientific and methodological level" at their disposal for use in universities and colleges of special secondary education.

- Following the recent press campaign against Academician Andrei D. Sakharov, the announcement that the Soviet government would not permit him to travel to Oslo to receive the Nobel Peace Prize came as no surprise. Nevertheless, the reason given—that he is in possession of classified information—demands some scrutiny. It is more than seven years since Sakharov lost his security clearance, which raises the question of whether any information to which he previously had access is still on the secret list.

Moreover, in 1958, the year that Boris Pasternak decided to decline the Nobel Prize for Literature after a prolonged press campaign against him, the award of the Physics Prize to Cherenkov, Frank and Tamm was hailed as a notable tribute to Soviet science, and the three winners received the necessary visas for Stockholm. This was in spite of the potentially sensitive nature of their work: Tamm and Sakharov had actually been working together on certain aspects of thermonuclear reactions.

The Soviet authorities apparently find Sakharov's prize an embarrassment, and for reasons of policy would prefer that he should not receive the award in person. If the precedent is followed in the future, however, a Soviet winner of the Physics Prize might well be forced to receive it *in absentia.*

- Increased concern is being felt for the fate of Andrei N. Tverdokhlebov, the physicist who was arrested on April 18 in connection with his attempts to establish a Moscow group of Amnesty International, and who is now being held in the Lefortovo prison in Moscow. The link between physics and concern for human rights and freedom dates back to Stalin's time, when the need for a nuclear arsenal allowed physicists to speak from a relatively privileged position and to avert a Lysenko-style disaster in the physical sciences. Tverdokhlebov has been closely associated with Academician Sakharov, whose "Human Rights Group" he joined as a founder member, and also with Valentin Turchin, who was a co-signatory of the second Sakharov manifesto of 1970. An appeal by Turchin on behalf of Tverdokhlebov has been sent to the West. According to press agency reports, Tverdokhlebov and another member of the Amnesty group, the Ukrainian science-fiction writer Mykola Rudenko, will be charged with "anti-Soviet slander", but it is not yet clear whether formal charges have been made.

- Two significant developments in the field of automation have been announced recently in the Latvian SSR. The first, described at the First All Union Conference on Biomechanics in Riga, is a proposed walking robot for rough terrain. Although this has been mooted several times in the last few years, the new version, designed on the basis of extensive mathematical simulation, seems to have been produced with far greater attention to practical implementation than some of the rather theoretical proposals so far discussed. The latest ideas include lasers as a possible means of stimulating vision, and the automatic selection of the form of motion —velocity, length and height of steps —to fit the demands of the terrain. A six-legged robot is likely to prove the most manoeuvrable.

The second and perhaps more alarming development concerns the management-control automation system recently introduced into the radio industry in Latvia. According to the *Novosti* agency, this system, based on a Minsk 22 computer, is intended to assess the competence of production managers.

Among other things, it checks their actual performance against planned deadline dates, and acts as a progress chaser by sending telex messages to remind managers of their obligations. At the end of the month, it evaluates the manager's place in the hierarchy of professional "emulation" and adjusts his pay accordingly.

The system, which operates with a staff of five, is said to repay the investment costs within a year by reducing inter-office correspondence by some 50%, as well as improve labour discipline. Its adoption has been recommended for all radio-engineering enterprises in the USSR. ☐

Another carcinogen?

from Laura Swaffield

THE problems of carcinogens in industry (see *Nature*, **258**, 94; 1975) are again highlighted by an article in the journal of the British Society for Social Responsibility in Science. The substance now implicated as a carcinogen is trichloroethylene, widely used as a degreaser and chemically similar to vinyl chloride monomer (VCM).

Its alleged link with humans will be harder to prove than with VCM, which is said to produce a rare form of cancer (angiosarcoma). And whatever the eventual findings, enforcement of controls may be difficult. Trichloroethylene is an important cause of gassing and is well known already as a narcotic with dramatic effects.

Precautions are laid down in the UK, though they are by no means always met, and it remains debatable whether enough attention has been paid in the UK to its longer term effects. □

Continuing crisis in Italian universities

from Gillian Boucher, Venice

AS the *Corriere della Sera* put it on November 5, "Today the universities are opening: Why?" The article, concerned with the colossal extent of graduate unemployment in Italy, touched on only one of the problems of the universities—problems that seem so daunting and so far from being soluble by the universities themselves that one marvels to see concerned professors still perfectly sane and conducting themselves in a manner not dissimilar from that of their colleagues in more fortunate countries.

But with the universities open to all who have passed the state school-leaving examination, laboratory and library facilities for teaching are hopelessly inadequate. It is not uncommon for professors to cope with their swollen classes by giving the same lecture three times in a morning. A much tougher entrance examination, even if it were politically acceptable, would not be a fair way of selecting students for science courses because of the bias of the school curriculum: Petrach and Dante loom larger than science for the average Italian schoolchild.

Many university teachers would welcome instead a tough examination at the end of the first year of university, but even that smacks too much of elitism to be politically acceptable at present. Fortunately many students drop out voluntarily. Last year at the University of Padua there were 2,500 first-year medical students, 1,000 in the third year and 500 in the sixth, final year.

Medical schools are in a particularly difficult position, for they have to compete for medically qualified staff with hospitals, which pay far better. The large pay increases recently awarded to hospital doctors mean that university departments often have to fill their junior posts with people lacking medical qualifications, further increasing the teaching load of medically qualified professors.

Difficulties in recruiting first-class graduates are not confined to medical departments. The chief problem is that since 1973 there have been created no more *assistenti*—junior lecturers waiting for a chair who have a certain amount of security and who consider themselves definitely part of the establishment. Now *assistenti* are being replaced with *contrattisti*, who have only a four-year contract and no certainty of a university career beyond that.

Restructuring at the top of the ladder is causing perhaps even more of a headache. The 3,000 top professors (known as *professori di ruolo*) have, with the growing student numbers, been aided by more and more assistant professors, or *professori incaricati*. Although this arrangement was obviously cheaper for the state, in 1973 it was finally decided that 7,500 new *professori di ruolo* should be created by competition: 2,500 in 1973–74, 2,500 in 1974–75, and a further 2,500 in 1975–76.

In the competition, a candidate's publications are assessed by a committee of *professori di ruolo* in the candidate's field, the committee's decision being subsequently blessed by the Ministry for Public Instruction and the university concerned. Unlike previous competitions of this sort, in which the committees used to be elected by all the *professori di ruolo* in a field, the names of the members of the committee are now picked out of a hat by the Minister for Public Instruction. This system has not prevented unconscionable delays in the formation of the committees—mostly caused by obstructive *professori di ruolo* who did not wish to see their privileges diluted by numbers.

The final and fundamental problem is that of money. Once the ministry has spent what it considers necessary for school and university teaching, the sum remaining for research is usually enough to pay for the animal houses and the telephone bill. University research is saved only by the Consiglio Nazionale delle Ricerche (CNR) which, as well as supporting autonomous institutes, funds institutes in university departments and provides grants for individuals.

Anyone lucky enough to be funded by the CNR can feel comparatively secure until 1980, the end of the coming quinquennium. But the CNR is moving towards a Rothschild-like attitude to research for the consumer and may become unwilling to fund projects which fall outside its own guidelines. If a new body being canvassed at present, a Ministry of Scientific Research, is ever created, the CNR would probably become its executive branch. Then, with research firmly separated from education, the CNR would no longer fund research in universities. And that would be the death of science in Italian universities. □

Eurospace body acts

from Angela Croome

IT hasn't taken long for the recently formed European *ad hoc* Committee for Space Science to go into action. Just a week after the European Science Foundation in Strasbourg agreed to its formation, two members of the committee attended a Washington meeting of the Space Sciences Board of the US National Academy of Sciences—the committee's American counterpart as a clearing house for long term research policy in the space sciences.

Active subjects on which high level international planning and cooperation are sought include the proposals for an International Planetary Decade being sponsored by the International Council of Scientific Unions' committee for space research, COSPAR, and the project for a large space telescope as an international research facility. A joint meeting of the American board and the European Committee on the telescope is expected before long.

There is likely to be a good deal of overlap between both the business and the membership of the European committee and a similar committee formed to concern itself with astronomy—though the latter has an initial lifetime of only a year, whereas the space sciences committee is to operate for two years.

The newly formed committee will want to keep an eye on the European Space Agency, established earlier this year, most of whose funds have gone on technology projects, principally satellite applications, rather than scientific research. The agency itself accepts that the balance is not right at present.

In the UK, meanwhile, the Director of Jodrell Bank, Sir Bernard Lovell, has said that the large area interferometer system financed through a £1.9 million grant from the Science Research Council is unmatched in its scale and sophistication. He also sees it

Lowering costs in higher education

KENNETH MELLANBY

When in 1947 I went to Nigeria to try to start that country's first university at Ibadan, I had a capital sum of £1.5 million provided by the British government for buildings. Tough negotiations persuaded the Nigerian government to increase its proposed annual grant (to meet other costs) from £60,000 to £100,000. These sums soon proved to be inadequate and larger amounts eventually became available; but until oil was discovered in the country we were always short of money.

I therefore find it hard to be as sympathetic as I should to the universities of the UK in their present financial position. After all, their grant from the goverment has increased by some 200 times in 25 years. I think that it is right that their expenditure should be carefully scrutinised.

There is growing concern about student numbers and the entry into different faculties. Politicians and others have suggested that money should not be spent on training specialists for jobs which do not exist, especially when the training is particularly expensive. Unfortunately, attempts to relate graduates to jobs have seldom been successful. Thus in medicine we had the Willink and Goodenough committees, which restricted the growth of medical schools at a time when expansion was later shown to have been needed.

Yet this should have been a comparatively easy exercise for, notwithstanding the egregious mistakes of our demographers (who in 1945 estimated that by 1965 Britain's population would fall by 4 millions, though in fact it increased by about the same number), the number of patients and their diseases remains fairly constant year by year. Few people would suggest at present reducing our intake of medical students, even though it costs the taxpayer something like £100,000 to train a qualified doctor.

Universities claim, with some justification, that they need to maintain a good academic balance, and that one faculty should not grow so rapidly that it overshadows the others. Some even believe that there is an ideal ratio for the number of students in different faculties. I remember hearing Lord Hailsham, when in charge of higher education some twelve years ago, deploring the insistence of universities on enlarging their faculties of law and social science, even when there was no increased demand for lawyers and social scientists, as a condition for producing the larger number of scientists for whom there seemed, at that time, to be a demand. Sadly, some of the increased number of scientists found, after graduation, that they were also unwanted. To estimate future manpower needs is clearly difficult.

When funds are plentiful, there is less demand to regulate entries into expensive courses. We are rightly proud of our high academic standards, and resist attempts to lower them. Nevertheless more thought could be given to reducing costs. If we are admitted to a British hospital, we will probably be attended by a doctor who qualified in India or Pakistan. Some may arrive with inadequate English and others (like, unfortunately, some of our own graduates) may be incompetent. But the majority carry out their duties admirably. Yet the cost of their training was only a fraction of that incurred in the UK. It is difficult not to believe that there is some room for economy, even in medicine.

Veterinary training is not quite so expensive, but costs probably amount to about £50.000 a graduate. This is justified because the veterinary surgeon makes an important contribution to food production by his care of farm animals. The present trend will double the number of vets in practice in Britain within the next 20 years. It is right to wonder whether this is wise or prudent. At present more than half of veterinary practice is devoted to what are, rather charmingly, called 'companion animals'. These are pets, dogs, cats and also horses and ponies. Now it would be wrong to suggest that these are unimportant. They bring pleasure to many, and companionship and solace to elderly and lonely people. They need to be cared for.

But it is surprising to find that some 6 million dogs, rather fewer cats and 250,000 horses take up more veterinary time than do 50 million farm animals (cattle, sheep and pigs) and nearly 150 million poultry. If our farm animals are receiving anything like proper attention, some companion animals would seem to be over-privileged, particularly as experience suggests that the only contact many dogs and cats have with a vet is to be put down when they are old or unwanted. If, as many expect, the number of intensively kept farm animals falls substantially as supplies of cheap imported feeding stuffs dry up, will we need as many vets for agriculture? Can we afford them for our pets?

It is generally agreed that all our school leavers able to profit from higher education should have the opportunity to do so, but need this be so expensive? Can we really justify a staff–student ratio three or more times as high as that obtaining in many other countries? Has enough attention been given to devising more general—and less expensive—courses? We often hear that change in science-based subjects is so rapid that every practitioner will have to be retrained at least once every 10 years. Are we devoting enough of our scarce resources to retraining, and might it not be better to reduce the scale of that given initially?

No doubt these problems are in many people's minds, but so far changes have generally raised rather than lowered academic costs. Perhaps the financial crisis will ensure that action is taken to reduce waste and still to maintain standards.

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as an important step towards the 375-foot radio dish planned for the observatory's Meiford site in Wales, some 85 km away.

The new facility, which will use three existing radio dishes that are capable of working down to centimetre wavelengths, will also increase the interferometer baseline from 20 to 85 km by adding a new 25 m low cost dish in Montgomeryshire. The whole system will be centrally controlled from Jodrell's new interferometer control room

"The new facility will not replace the 'big dish'—it simply makes the best use of the equipment and expertise we already have", says Sir Bernard. As for the 'big dish', he still hopes to finance the 375-footer at Meifod. Plans had to be dropped two years or so ago because the international tenders were far higher than expected: £16 million as against an estimated £10 million □

correspondence

Computer prehistory

Sir,—Professor Wilkes (October 16) identifies the prehistory of computers with "the life's work of just one man, Charles Babbage (1792–1871)". If Babbage's work is likened to the prehistory of the subject, how is one to classify the elaboration of the real first arithmetical machine, almost two centuries earlier, by Blaise Pascal?

Pascal invented and built his arithmetical machine in 1643, at the age of 19, and obtained a patent for it (*Privilège pour la Machine d'Arithmétique de M. Pascal*) in 1654, at which time he had already constructed more than 50 different models. Although Babbage wrote in rather denigrating terms of "the contrivances of Pascal and others" (which actually worked) as compared to his own engines (which did not), Pascal's invention was indeed fundamental, and the actual precursor of all mechanical computers, including Babbage's.

A number of Pascal's machines are still in existence, for example, at the Conservatoire des Arts et Métiers in Paris. A detailed description of the mechanism and principles of Pascal's arithmetical machine was published in 1751

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EEC directives

Sir,—As a member of the Conservation Society since its inception, and being much concerned about pollution, I would like to support Eric Ashby (October 16) against Paul French (November 6) The latter may be right in his final statement to the effect that other Europeans are more willing to tackle pollution than are the British. As a result of their efforts, for all I know, the Rhine may now, like the Thames, have more fish in its lower reaches than it has had for 150 years.

But his insistence that identical risks should apply everywhere is absolutely unreasonable. In the denser parts of big cities a complete ban on the burning of smoky fuels has reduced by several times the level of smoke pollution. It would not have been sensible to apply the same rules to villages and isolated farms in the country. Not only would uniform laws for town and country have led to a large extra cost for little useful achievement, but, much more seriously, such laws would never have been passed. I do not believe that in any country in Europe costly and novel proposals, however valuable in the long run, will fail to arouse some opposition. If they can be shown to be technically unreasonable over large areas, opposition will be much greater.

I can see that administrators like to have a uniformly applicable formula, against which performance can be judged. The new formula, which says that pollution is equally important everywhere, is a real advance on the previous non-formula which in effect said that most pollution was of no importance whatever.

It would not seem any more difficult, however, to use a formula which recognised that pollution is more important if more people are polluted. One would do better with a formula based on the square of this number, since if an area has twice as many people they are likely to produce twice as much pollution and there are twice as many to suffer this double pollution. A linear system (the importance of pollution being finite for zero population) would be much easier however, as the standard effluent permitted in any area could then be fixed only in terms of the slowly changing population in the affected area and not at all by the statistically larger fluctuations of the numbers of polluting factories.

Like Mr French, I would be entirely in favour of Britain spending £100 million a year on reducing pollution (and simultaneously, perhaps, unemployment). But to spend this on reducing pollution in the Atlantic Ocean rather than in the thousands of kilometres of rivers in which no fish can live would be a technical and economic stupidity that has nothing to do with politics or nationalism.

John H. Fremlin

University of Birmingham, UK

Sir,—I am not prepared to accept the whole of Lord Ashby's thesis, contained in his comments on the EEC pollution debate. He states, for instance, that "rabid conservationists need to be reminded sometimes that all pollution, except that from atomic weapons, is a by-product of processes which benefit society" I would have thought that conservationists—rabid or otherwise—are merely saying that any process which endangers the environment for future generations cannot possibly be regarded as beneficial to society.

Again it seems to me complacent, to say the least, to accept a report published by the Medical Research Council 16 years ago as a justification for pouring more sewage on to our beaches and noxious chemicals into our rivers, even though myriads of microbes may be working late into the night to lessen this evil.

Lord Ashby also refers to the phoney arguments. What can possibly be more phoney than his own argument that a cost of £100 million to reduce pollution is prohibitive when we spend many more millions every year on luxuries such as gambling?

W. J. Davies

Tenby, UK

Offshore structures

Sir,—We have had some experience with modelling offshore structures in the laboratory, and wish to call attention to the need for better understanding of the engineering problems likely to arise as drillings are made in deeper and deeper water. Mishaps that have already occurred have been serious enough, but the failure of one of the giant rigs or platforms now being planned would be a catastrophe of unprecedented proportions.

Waves not only exert the major load, which is horizontal and fluctuating, but they also affect the sea bed under and around the structure. The interaction is complicated and difficult to model, but studies and fragmentary prototype data have provided some clues. The foundation and anchorage problems assume greater importance as structures grow in depth.

Although some research seems to be in progress, there is little doubt that it has only just commenced. The present programmes are carried out for private industry whereas research on hydraulic phenomena was formerly for government agencies. Past results were thus public, but the present research is mainly *ad hoc* and confidential, in the long term being more costly to all concerned. Reasons for mishaps now tend to be kept secret for political or commercial reasons.

Basic issues should be investigated openly by university and similar institutions, if only so that controlling authorities and insurance agencies can establish design criteria.

Chesley J. Posey

University of Connecticut

Richard Silvester

University of Western Australia

news and views

Histocompatibility antigens and immunoglobulins?

from Alan F. Williams

In higher vertebrates there is a chromosomal region which contains closely-linked genes for histocompatibility antigens, and also genes which regulate immune responses. The major histocompatibility antigens which are recognised by antibody are called H-2 (K and D) and HLA (-A and -B) antigens in mice and men respectively, and seem to be coded for by two loci which are polymorphic within a species. The function of histocompatibility antigens is unknown, but the finding of genes controlling immune function in the same chromosomal region has led to the idea that histocompatibility antigens may also be involved in tissue interactions as cell-surface receptors. Taking this idea further, it has been suggested that these antigens may be related evolutionarily to immunoglobulin (see Bodmer, *Nature,* **237,** 139; 1972; Schreffler and David, *Adv. Immun.,* **20,** 125; 1975, for reviews).

Recent biochemical studies on histocompatibility antigens of mouse, man and rat have shown that they are carried by the polypeptide chain of a cell-surface glycoprotein of about 45,000 molecular weight, and that closely associated with this, but not covalently bound, is another polypeptide of about 12,000 molecular weight which is not polymorphic. This latter molecule is β_2-microglobulin which shows amino acid sequence homology with immunoglobulin (see *Transplantn Rev.,* **21,** 1974). These findings have been interpreted as supporting the idea of a receptor function for histocompatibility antigen, and also the possibility that the 45,000 glycoprotein is related to immunoglobulin. In fact such is the euphoria which has been engendered, that virtually every product of genes linked to the histocompatibility antigen region is now being considered to be related to immunoglobulin (for example Capra *et al., J. exp. Med.,* **142,** 664; 1975). This all involves a number of extrapolations and it is relevant that neither immunoglobulin nor β_2-microglobulin genes are linked to the histocompatibility antigen region (see the reviews cited above and Goodfellow *et al., Nature,* **254,** 267; 1975)

It is obvious that the relationship between histocompatibility antigen and immunoglobulin can only be determined by comparison of amino acid sequences, but in lieu of such data more circumstantial similarities have been sought. Several workers (Strominger *et al., Transplantn Rev.,* **21,** 126; 1974; Cresswell and Dawson, *J. Immun.,* **114,** 523; 1975; Peterson *et al., Proc. natn. Acad. Sci. U.S.A.,* **72,** 1612; 1975) have suggested that histocompatibility antigens are composed of a four-chain structure of two identical 45,000 glycoprotein molecules plus two molecules of β_2-microglobulin linked by disulphide bonds between the large molecules. This is based on the apparent size of histocompatibility antigen in detergents determined from their hydrodynamic properties, and also by the finding of bands in SDS polyacrylamide gel electrophoresis which appear to be dimers of the large subunit. Other evidence cited as establishing a link between histocompatibility antigen and immunoglobulin is that the antigens may contain intrachain disulphide bonds like those in immunoglobulin, and that histocompatibility antigen has affinity for a bacterial product—staphylococcal protein A, which binds to immunoglobulin. One might describe the evidence for all these points as tentative, and interpretations relating to the size of the antigens in detergent may be incorrect. Thus Snary *et al.* (page 240 of this issue of *Nature*) have produced evidence suggesting that the molecular weight of HLA antigen in deoxycholate is incompatible with a four-chain structure.

There are difficulties in estimating the size of membrane molecules in non-dissociating conditions. Integral membrane proteins are usually solubilised by detergents of which they bind large amounts (Helenius and Simons, *Biochim. biophys. Acta,* **415,** 29; 1975). Tanford *et al.* (*Biochemistry,* **13,** 2369; 1974) have described methods for accurate estimates of molecular weight in detergent and from chemical composition, measurements of detergent binding, and sedimentation equilibrium data, molecular weights can be calculated. For this, milligram amounts of pure material are needed while at present, estimates of the size of histocompatibility antigens can only be made with detergent extracts of impure material. Under these conditions only the size of the detergent–antigen complex can be estimated. This is derived from measurements of Stoke's radius by gel filtration, and sedimentation coefficient and partial specific volume ($\bar{v}$) by zone sedimentation on sucrose gradients in H_2O and ^2H_2O (Meunier *et al., FEBS Lett.,* **24,** 63; 1972; Clarke, *J. biol. Chem.,* **250,** 5459; 1975). Analysis by gel filtration and sedimentation on sucrose gradients in H_2O is a minimal requirement and large errors will result, particularly if gel filtration only is used since most membrane proteins including cell-surface antigens behave as if asymmetric in detergents (Letarte-Muirhead *et al., Biochem. J.,* **143,** 51; 1974: Snary *et al., op. cit.;* Clarke, *op. cit.*). A $\bar{v}$ value is often assumed rather than measured, and this is reasonably valid if the detergent used is deoxycholate since this has a v of 0.76 ml g^{-1}, close to that of proteins and glycoproteins (0.7–0.75 ml g^{-1}). With non-ionic detergents whose $\bar{v}$ is about 0.9 ml g^{-1} a valid assumption of $\bar{v}$ cannot be made without detergent binding data.

Snary *et al.* solubilised HLA in deoxycholate and carried out gel filtration and zone sedimentation on sucrose gradients; a $\bar{v}$ value was assumed. From this, molecular weights which include bound deoxycholate of 88,000 and 78,000 respectively were obtained for A and B series HLA antigens. Similar data has been obtained for rat histocompatibility antigen in deoxycholate and the molecular weight was estimated to be 100,000 (Letarte-Muirhead *et al., op. cit.*). These values are more compatible with a two-chain structure plus bound detergent (minimum size 57,000), than a four-chain structure (minimum 114,000). Peterson *et al.* (*op. cit.*) estimated the molecular weight of HLA and H-2 in the non-ionic detergent NP-40 to be 130,000. This would be compatible with a four-chain structure with little detergent bound, or more likely a two-chain structure plus a large amount of bound NP-40.

If the antigen-detergent complex does not contain a four-chain structure then there is the difficulty of explaining the reason for the appearance of the 45,000 chain in SDS polyacrylamide gel electrophoresis. Snary *et al.* have suggested that this may be due to disulphide exchange either in stored membrane, or possibly during immunoprecipitation procedures. Clearly the experiments must be repeated in the presence of oxidising agents.

If one accepts that histocompatibility antigens may have a two-chain rather than a four-chain structure does this argue against a relationship between the antigens and immunoglobulin? Obviously not, and this merely points up the circumstantial nature of the current evidence. The matter can only be settled by amino acid sequence analysis of the 45,000 molecular weight glycoprotein which carries the antigens, and comparison of this with immunoglobulin sequences. Given the current progress in the purification of HLA and H-2, and also in microsequencing techniques, such data should be forthcoming in the next few years. ☐

Chemical language in insects

from our Insect Physiology Correspondent

It has long been known that scents produced by glandular organs play an important part in animal communication. The naming of such chemicals, used for communication between members of the same species, as 'pheromones' by Karlson and Lüscher coincided with the development of gas liquid chromatography, mass spectrometry and other methods which have made possible the chemical analysis of minimal quantities of material. The result has been a very rapid expansion of knowledge in this sphere. Whereas it took Butenandt and his associates many years and vast amounts of material to identify the chemical structure of 'bombycol', the female sex attractant of the silkworm moth, by classical procedures, complex mixtures can now be analysed in a matter of days

The chemicals present in the glandular secretions of insects are often exceedingly diverse; 20-30 recognised components in a single gland. They comprise a vast range of saturated and unsaturated hydrocarbons and their derived alcohols and aldehydes (ranging from hexenal and tridecene to pentadecane), fatty acids (such as butyric and caproic acids), a great number of phenolic substances and quinones (such as phenol, *m*-cresol, benzoquinone and toluquinone) and a variety of terpenoids (such as geraniol, farnesol, citral, and citronellol).

So far as the perceptions of man are concerned, in recognising the odours characteristic of particular kinds of shops, or appreciating the bouquet of a vintage wine, it seems to be the complex mixture which is assessed. Insects seem often to be concerned with a single component: in the mandibular gland secretion of the queen honey bee (as shown by Butler and his associates) there are more than 30 compounds; of these, 9-ketodecenoic acid is the 'queen substance' which attracts males during the nuptial flight and inhibits royal-cell building by workers in the nest, whereas 9-hydroxydecenoic acid causes clustering and stabilisation of worker swarms.

The action of pheromones is commonly divided into two classes (following Wilson and Bossert): chemical 'releasers' of specific acts of behaviour, and 'primers' which seem to act initially on the endocrine system The same pheromone may show both types of activity—as in the case of the queen substance which, besides its effects on behaviour, inhibits ovarian development in the workers. Moreover the same chemical can be used both as a pheromone for communication within the species and as an 'allomone' (as defined by Brown and Eisner) to provide defence against an enemy. That combination is well seen in the use of formic acid by the wood ant. Thus the effects of a pheromone depend on circumstances, on differences in concentration, and in some instances on the combination of secretions from different glands. It has even been suggested by Bossert that concentrations might be modulated in time. It is unlikely that the modulation of odour emission could attain the level of a symbolical or syntactical language but as E. O Wilson points out the acoustical system has acquired quite complex signal modulation in the songs of insects.

The signal provided by a pheromone is the 'active space' as defined by Bossert and Wilson· the zone around the point of emission within which the concentration of pheromone molecules is above the threshold level of response. This zone may vary in shape with movement of the air, and in composition depending on the comparative rates of diffusion of the varied molecules. As Wilson has pointed out "the relation of chemosensory physiology to ecology can be fully elucidated only through the analysis of the active spaces".

These ideas have been carried some steps further by interesting observations of Bradshaw, Baker and Howse on the multicomponent pheromones of the weaver ant *Oecophylla*, as described on page 230 of this issue of *Nature*. These authors find evidence of caste differences in the mandibular gland secretions as well as differences in the components to which the castes respond. Furthermore, they have made interesting advances in studying the changes in the 'active spaces' of particular components which appear to provide sequential messages determined by the volatility of materials and the threshold concentrations for different responses. Worked out in detail in relation to the mode of life of a polymorphic species, all this shows the immense potentialities of chemical communication in the regulation of behaviour in a social insect. ☐

Fibroblast model for hormone effects

from Robert Shields

One of the most striking features of the action of trophic hormones on cells is that many of the resulting changes in metabolism are remarkably similar, regardless of the type of hormone or the target tissue. Thus changes produced by oestrogen in the uterus, corticosteroids in the liver and insulin in muscle are much the same. These changes include alterations in macromolecular synthesis and a great deal of endocrinological research has been directed at finding out how hormones regulate these processes. The classical endocrinological approach has been to remove the gland producing the hormone under study and after a suitable period re-add the hormone and follow the subsequent changes in the target tissue. Although much qualitative data has been gathered in this way, the lack of suitable *in vitro* systems has hampered a quantitative assessment of the inter-relationship between the hormone-induced changes in RNA, protein and DNA synthesis.

One well known *in vitro* system that has been studied intensively (but for rather different reasons) is the control of growth of fibroblasts in tissue culture. It now seems apparent that the growth of these cells is regulated by the levels of serum factors available to the cell (*News and Views,*

253, 689; 1975) and that these factors are probably hormones which have not yet been fully characterised. Decreasing the availability of serum to cultured cells either by reducing the serum concentrations or allowing the cells to reach confluence is the *in vitro* equivalent of removing the gland producing the trophic hormone, re-adding the serum is the equivalent of hormone replacement Since the changes in metabolism induced in serum-stimulated fibroblasts are very similar to those produced by trophic hormones (Hershko *et al.*, *Nature new Biol.*, **232**, 206; 1971), a study of the changes induced in these cells may give clues as to how trophic hormones operate *in vivo*.

Rates of macromolecular synthesis are almost invariably assayed by incorporation of radioactive precursors, and the accuracy with which the incorporation rate reflects rates of synthesis is open to question. An alternative indirect approach depends on assaying steady state concentrations and rates of degradation of macromolecules. The inferred rates of synthesis can then be compared with those from tracer studies. By this method it has been deduced that growing fibroblasts have about four times the protein synthetic rate per unit protein than non-growing cells and differences of this order of magnitude have indeed been suggested by labelling studies (Rudland, *Proc natn. Acad. Sci. U.S A.*, **71**, 750; 1974). In principle this approximately four-fold increase in protein synthetic rate could be produced by controlling the levels of cytoplasmic mRNAs and/or controlling the rates at which the mRNAs are translated. The question as to which of these events is dominant in the hormonal stimulation of protein synthesis has long been keenly debated, the cultured fibroblast provides an ideal system for measuring their relative importance.

It is conceivable that the translation of mRNA into protein could be governed by the availability of ribosomes. In growing cells rRNA appears to be stable (Weber, *Nature new Biol.*, **235**, 58; 1972; Abelson *et al*, *Cell*, **1**, 161; 1974), but in non-growing cells the rRNA turns over at about 1% per h This gives a relative rate of rRNA synthesis in growing compared with non-growing cells of about four-fold based on their relative RNA content, or about eight-fold on a per cell basis. Cells which have just been stimulated would have intermediate rates of rRNA synthesis, and this has been confirmed by labelling experiments (Bandman and Gurney, *Expl Cell Res.*, **90**, 159; 1975; Rudland, *op. cit.*). How these relatively large differences in the rates of rRNA synthesis are achieved is not yet clear. Certainly increased transcription of rRNA genes occurs (Emerson, *Nature new Biol*, **232**, 101, 1971; Mauck and Green, *Proc. natn. Acad Sci. U.S.A.*, **70**, 2819; 1973) but whether there is also less wastage of rRNA precursors as seen in stimulated lymphocytes (Cooper, *Nature*, **227**, 1105, 1970) is not known. But although the increased rate of ribosome synthesis in cells recently stimulated can hardly have any impact on the total ribosome content for some hours, the increased rate of protein synthesis is observed very rapidly. This must mean that some other feature of the protein synthetic machinery is altered

Keen proponents of the applicability of the Jacob-Monod model to mammalian systems might suggest that an increase in mRNA content is responsible. In both growing and resting cells the half-life of mRNA is the same (Abelson, *op. cit*) and corresponds to a degradation rate of 8% per h This would mean that the relative rate of mRNA synthesis in growing and resting cells was 1.4 times their relative mRNA contents Here there is some conflict in the literature Using steady state labelling Johnson and his coworkers (*Cell*, **1**, 95, 1974) claim that resting cells contain two to four times as much mRNA on a per cell basis as resting cells On the other hand, using a method of quantitating mRNA by hybridising ^{3}H-poly(U) to the poly(A) tracts of mRNA, Rudland and his collaborators found at the most a 30% increase in growing cells (*J. molec. Biol*, **96**, 745; 1975). The former estimate would give a relative rate of mRNA synthesis of three to six-fold on a per cell basis, the latter a 1.6 to 1.8-fold increase in growing cells. In fact, analysis of the rates of appearance in the cytoplasm of ^{3}H-poly(A) tracts attached to mRNA gives a relative rate of 1.8-fold in stimulated cells (Bandman and Gurney, *op cit.*), suggesting that the relative mRNA contents of growing cells may be only marginally higher than in resting cells. One unexpected result from these experiments is that the increase in mRNA synthesis seen in growing cells is not due to an increase in gene transcription but rather to an approximately two-fold increase in the efficiency with which mRNA molecules are clipped from their high molecular weight precursors in the nucleus (Johnson *et al*, *Cell*, **4**, 69; 1975). In any event, the small increase in mRNA content in growing cells is not sufficient to account for the approximately four-fold increase in the rates of protein synthesis seen in stimulated cells, especially since the increase is so rapid.

When the cytoplasmic location of poly(A)-containing mRNA in non-growing cells was examined, it was found that a significant amount of mRNA, variously estimated at between 37% (Bandman and Gurney, *op. cit.*) and 85% (Rudland *et al.*, *op cit.*) was not associated with polysomes, but was in the form of slowly sedimenting ribonucleoprotein particles On stimulation this mRNA was rapidly recruited into polysomes which are formed at the expense of free ribosomes. A number of indirect experiments show that the existence of mRNA in these particles is due to a low rate of initiation of ribosomes on mRNA After stimulation this rate is rapidly increased (Rudland *et al.*, *op. cit.*; Bandman and Gurney, *op. cit.*). It seems that the increased rate of initiation of ribosomes on mRNA and the resulting recruitment of pre-existing untranslated mRNA is sufficient to account for the rapid increase in protein synthesis seen after serum stimulation. Later, the extra concentration of mRNA and ribosomes will help maintain the high rates of synthesis.

The conclusion reached from these results is that the substantial changes in the general rate of protein synthesis seen in stimulated cells are governed largely at the translational level rather than at the level of mRNA transcription Gratifyingly, similar conclusions have been reached using much more indirect experiments for the action of trophic hormones *in vivo* (Korner, *Ciba Foundation Symposium*, **86**, 1970). Induction of specific proteins in animal cells however such as the induction of oralbumin by oestrogen in the Chick oviduct, may rely more on changes in the amount of specific mRNAs. ☐

Man against nature in the climatic stakes

from John Gribbin

LATEST indications suggest that all of man's polluting influences on climate may be acting to produce a global warming, while the natural trend extrapolated from past variations is towards a cooler regime. In the short term, the former influence may well dominate, and this suggests the need for a radical rethinking of some predictions, such as those concerned with the impact of climatic change on agriculture and world food production.

Until recently, it could be argued that some aspects of man's activity cause a warming tendency (CO_2 and the greenhouse effect) while others tend to cool the Earth (atmospheric dust) so that on balance they might be ignored. But last year several calculations were published showing that the net effect of the aerosol dust particles might also be to cause a global warming (*Nature*, **253**, 162; 1975). More recently, R. A. Reck has suggested that the present background aerosol density should be contributing a temperature increase of 0.2 K at 85°S and 0.05 K at 85°N, acting to oppose the increase in snow and ice cover in 1971 which some climatologists have linked with circulation changes associated with the Sahelian droughts of the early 1970s (*Science*, **188**, 728; 1975). The difference in the size of this effect in the two hemispheres arises because it depends on the relative albedos of the aerosols and the underlying surface, and there is of course much more land in the north.

So what of the greenhouse effect? Considering CO_2 alone, and ignoring any effects due to dust, W. S. Broecker has suggested that a pronounced warming could become apparent within a decade (*Science*, **188**, 460; 1975) There have been many calculations of the direct warming which would be caused by a specific increase in CO_2 content of the atmosphere, and although these have not always agreed with one another the differences between them are now small enough for Broecker to produce a plausible extrapolation of the exponential growth of this contribution to global temperature up to the end of this century. Where his study goes a step further than those of most workers is in combining this warming trend with an extrapolation of the 'natural' trend of temperature from the pseudo-periodic fluctuations of the ^{18}O record in the Camp Century ice core.

The combined effect of the two processes is likely, on this model, to produce dramatic changes in the near future. The natural cooling phase since the mid-twentieth century has been opposed by the growing greenhouse effect, the net result being only a modest cooling; but according to the ice core evidence, this trend is likely to reverse within ten years, and from then until the end of this century the natural trend and the greenhouse effect will combine to produce a pronounced warming, increasing mean global temperatures by half a degree or more above the present level. According to Broecker, the greenhouse effect would then be operating so strongly that future natural cycles with the 80-year Camp Century 'period' would serve only to modulate the ever steepening rise in temperature. This, he says, "by early in the next century will have driven the mean planetary temperatures beyond the limits experienced during the last 1,000 years". In fact, that may be no bad thing, since the past millenium has been, by and large, cold and unsuitable for the world to support anything like the population expected for the end of this century. But as Broecker stresses, the agricultural consequences of such a warming are not obvious, and we do not know how a change in mean global temperature would be likely to affect rainfall patterns in the all important grain producing regions.

Some aspects of Broecker's study are certainly speculative, but none the less stimulating in highlighting the need to consider the possible impact of such a warming, and not to be too restricted and look only at the problems posed by cooling trends. The need to avoid the stereotyped approach is also highlighted by the discovery that chlorofluorocarbons—familiar to doomwatchers as possible destroyers of the ozone layer—may also contribute directly to climatic change, again through a greenhouse effect.

These compounds are strongly absorbing at infrared frequencies, and V. Ramanathan calculates (*Science*, **190**, 50; 1975) that if the concentration of them in the atmosphere reaches two parts per thousand million there could be a corresponding increase in surface mean temperature of more than 0.25 K. This is the concentration which may be reached by the year 2000 if present levels of injection continue (Molina and Rowaland, *Nature*, **249**, 810; 1974), and although smaller than the projected CO_2 influence this would be significant alone. With this effect and perhaps the aerosols adding to the CO_2 effect there seems nothing to oppose the warming influence of man's polluting activities except the natural variations of climate.

Those natural variations must dominate in the long term, and even within a hundred years of CO_2 production at the rate implied by Broecker's extrapolation there will be little prospect of further significant increases since there will be little fossil fuel left to burn. So the pronounced global warming he envisages is, even if genuine, only likely to be a short term phenomenon, assuming that plants continue to take up CO_2 from the air. Looking further ahead to the next 1,000 years (which is a short enough span in climatic terms), the overall cooling tendency of the globe is likely to continue into a full ice age, according to studies of isotope variations in cores drilled from the floor of the Antarctic sea. These studies, made by a team at Lamont-Doherty Geological Observatory, were reported to the recent Norwich conference on climate by J. Hays.

Hays' interpretation of long term climatic fluctuations from this evidence provides strong support for the Milankovitch theory that changes in the tilt and precession of the Earth affect insolation at critical latitudes sufficiently to cause ice ages, and suggests that while ice ages are roughly synchronous in the two hemispheres the south leads the north by a few hundred or a thousand years. Since the most recent sediments show isotope variations implying that the southern hemisphere has already begun to move towards a new ice age, the conclusion that a full global ice age is due within a millenium follows inevitably from this interpretation of the past variations. In that case, and looking at the lifetime of our civilisation rather than that of an individual, it seems we should make the most of any warm spell we do get in the coming decades, since there is a long cold spell to follow. □

Ecological carousel

from Peter D. Moore

ECOLOGISTS, like the Greeks, are seekers after ideals. Among conceptual ideals, none is less tangible, or more sought after, than that of the ecological climax. The idea of the perfectly stable, equilibrium ecosystem which has developed through a sequential series of successional stages, is a very attractive one, but even as the concept evolved in the minds of such men as Clements, it gave rise to ecological controversy. The Clementsian ideal of a climax system in equilibrium with climate was soon demonstrated to be impracticable when applied by Tansley to vegetational studies within the British Isles. Tansley insisted that stability was the essence of climax and therefore any overriding factor of the environment, be it edaphic, topographic or biotic, which limits the development of an ecosystem short of its potential climatic climax state, can be the determinant of a climax system.

The development of ecosystem theory in recent years has necessitated a restatement of the climax ideal, and this has been provided by Odum (*Science*, **164**, 262; 1969), who based his definition on the characteristic energy flow and nutrient cycling patterns to be expected from a steady-state system. In practice, however, as in the case of the Clements–Tansley debate, much depends upon the scale, both temporal and spatial, on which any system is being studied. Apart from natural calamities, which are of frequent occurrence in many ecosystems (see Loucks, *Am Zool*, **10**, 17; 1970), the inevitable death and collapse of climax dominants, particularly in forest ecosystems, will result in renewed succession with a frequency dependent upon the generation time of the dominant. Consequently, the stable system found in the real world consists of a mosaic of microsites each of which may be in a different state of successional development or dominant maturation. The whole system may, nevertheless, approximate to the Odum ideal in terms of its energy and nutrient balance sheet.

This state of affairs is well documented in a recent study by Forcier (*Science*, **189**, 808; 1975) of the canopy tree species in a forest in New Hampshire. Within this forest, 90% of the trees belonged to one of three species, namely beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*) and yellow birch (*Betula alleghaniensis*). Forcier observed 400 randomly chosen plots within this forest over a period of three years and paid particular attention to the population dynamics of the seedlings of these species. Mortality of beech seedlings was found to be high initially but declined with age, thus average life-expectancy increased. Birch, on the other hand, had a fairly constant mortality whatever the seedling age. Sugar maple was intermediate in its population dynamics. Also, beech seedlings were found to vary little in their density whilst birch seedling densities varied considerably and average density was more than five times that of beech. These data suggest that birch is behaving as an opportunist in the composition of the forest.

Association analyses between the seedlings and the canopy trees showed that sugar-maple seedlings were associated with birch canopies and beech seedlings with sugar maple canopies. Evidently the three species are related in a cyclic successional series in which disturbance (or death of a mature tree) leads to birch invasion, sugar maple fares well beneath the birch canopy, eventually replacing it, and finally beech invades and replaces sugar maple. The roughly equal abundance of the three species of tree in the canopy is indicative of the importance of this cyclic microsuccession in what appears superficially to be a stable, 'climax' forest.

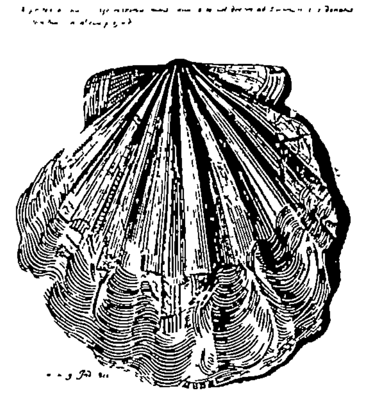
Forcier calls this a 'near climax ecosystem', but this is rather misleading, since it suggests that the system falls short of the ideal. In a sense it does, for the community consists of a mosaic of sites, most of which are in different stages of the cyclic development. Nevertheless, the forest as a whole complies with many of the demands of the Odum ideal. There is no net increase in biomass, so gross ecosystem production is equalled by ecosystem respiration Also the total input of nutrients from all sources (meteorological, geological and biological) must be equalled by total outputs. Reality fails to live up to the ideal only in that the 'stable' system contains species which cannot regenerate efficiently beneath their own canopy and which one therefore tends to associate with successional stages instead of the climax. Rather than call this forest a 'near climax', let us be ready to modify further the climax dogma and admit that climax is a carousel. ☐

What drives the solar cycle?

from Nigel Weiss

IAU Symposium No. 71 on Basic Mechanisms of Solar Activity was held in Prague on August 25–29, 1975.

THE Sun is the only star that can be observed in detail and any theory of stellar structure must be tested against these observations. Unfortunately, conventional models cannot explain the low measured neutrino flux or the excitation of oscillations in the Sun. as I. W Roxburgh (Queen Mary College, London) put it, "solar physics has forced the stellar physicist to think again." Over the past few years solar physics has developed, rapidly Observationally, it has become possible to resolve structures with scales smaller than the photosphere granulation, while theory is advancing from simple mechanisms to detailed computations.



Reproduction of the illustration of the earliest figured and described fossil from America. From Lister's (1687) *Historiae Conchyliorum Liber III.* Lister's entire description is with each figure and not in a separate text. Say (1824) mentions this figure as being his species *Pecten jeffersonius.* (Reproduced from *"Chesapecten,* a new genus of Pectinidae (Mollusca: Bivalvia) from the Miocene and Pliocene of Eastern North America"—*Geol. Surv. Prof. Pap. 861*

The solar cycle is apparently typical of stars with convective envelopes and O. C. Wilson (Hale Observatories) has found similar behaviour in several late-type stars. Magnetic fields on the Sun have been observed in profuse detail, from satellites and from the ground; indeed, we have far less information about fields in the Earth's core, which is shielded by the mantle. It is generally accepted that the cycle is maintained by an oscillatory dynamo yet E N. Parker (University of Chicago) emphasised that many features of this process are still poorly understood.

The orthodox model is a dynamo driven by a combination of differential rotation and helical motion in the outer convective zone. A dipole-like field is wound round the Sun by differential rotation and the resulting toroidal field is twisted by helical convection to produce a reversed poloidal field. Samples, particularly of the strong toroidal field, pop through the surface in active regions. This process can be represented by mean field electrodynamics: F. Krause and K.-H. Rädler (Zentralinstitut für Astrophysik, Potsdam) described the procedure of separating large scale mean fields from small scale fluctuating fields and then averaging over space or time Turbulent convection provides an enhanced eddy diffusivity and, through helicity (the α -effect), generates the poloidal field. Turbulence also produces fluctuating fields which, in the Sun, may be 100 times greater than the mean field itself. M. Stix (Göttingen University) discussed numerical solutions of the linear dynamo equations. With carefully chosen distributions of helicity and angular velocity, these yield remarkably accurate reproductions of the actual solar cycle.

Mean field electrodynamics is not perfect, however. The growth of the field is limited by non-linear effects that are not properly incorporated in the theory Moreover, the procedure of averaging and smoothing cannot be justified for giant convective cells and highly concentrated fields. J. O. Stenflo (Lund University) has established that more than 90% of the photospheric magnetic flux is concentrated into small regions, a few hundred kilometres across, with fields of around 1.500 gauss. Outside these regions, except in pores and sunspots, only weak, irregular fields are detected. To describe such a jagged field distribution, a more elaborate theory is needed.

Unfortunately, the pattern of motion in the deep convective zone remains unclear. R. Howard and H. Yoshimura (Hale Observatories) have accumulated Doppler measurements of the differential rotation which also provide some evidence for the existence of giant cells extending through the whole depth of the convective zone W. J. Wagner (Sacramento Peak Observatory) showed that coronal holes have an almost constant rotation rate which, with the sector structure of interplanetary fields, suggests the presence of cells elongated parallel to the rotation axis of the Sun. There is as yet no adequate theory of vigorous convection in a compressible fluid, though the interaction between convection and rotation has been studied for incompressible fluids. B. R. Durney (National Center for Atmospheric Research, Boulder, Colorado) found that the observed uniformity of the energy flux at the solar surface seemed incompatible with an angular velocity that increases inwards (as required for dynamo models). P. A. Gilman (NCAR) described numerical experiments on convection in a rotating spherical shell which led to large variations of angular velocity, though not necessarily to an equatorial acceleration. It is through such large scale computations that further theoretical progress must be made.

Turbulent diffusion is an essential feature of any solar dynamo, though there is no complete theory for the reconnection of magnetic fields on a sufficiently rapid time scale. The generally accepted view is that turbulence can bring opposing fields close enough together for dynamically driven reconnection to take place. E. R. Priest (St. Andrew's University) and A. M. Soward (Newcastle University) demonstrated that rapid reconnection can occur at rates up to the Alfvèn speed. J. H. Piddington (CSIRO, Sydney) dissented from the orthodox approach. He believes that turbulence may redistribute magnetic flux but is unable to destroy it. Indeed, his magnetic fields form isolated flux ropes that control the pattern of cellular convection (instead of being concentrated by the motion). So he has revived an alternative model of the solar cycle, relying on a primordial steady poloidal field from which the alternating toroidal field is generated by some form of torsional oscillation.

Immunological tolerance

from a Correspondent

> A symposium on Immunological Tolerance was held at Reinhardsbrunn, GDR on October 14–17. It was sponsored by the International Union of Immunological Societies and the World Health Organization.

THE mechanisms of antigen-induced unresponsiveness (immunological tolerance) are still controversial: tolerance has been ascribed either to "deletion" of relevant lymphocyte clones, or to active suppression of these clones by other lymphocytes (T suppressor cells) or by humoral "blocking factors".

The classical concept of clonal deletion in tolerance to transplantation antigens, first propounded by Sir MacFarlane Burnet and others, has withstood the test of time. Experiments by H. von Boehmer and J. Sprent (Basel Institute for Immunology) with mouse radiation chimaeras (F_1 hybrid mice reconstituted with bone marrow cells from both parents), provide clear evidence that self-tolerance in these animals is due to clonal elimination and not to suppression.

Evidence for blocking factors in the maintenance of tolerance was also presented, however. H. Wekerle (Max Planck Institut, Freiburg) has induced autoreactive lymphocytes by coculturing lymph node cells and testicular cells from the same rat. The cytostatic activity of these cells is blocked by an unknown factor present in autologous serum, and to a lesser degree by serum from allogeneic rats. M. Embleton (Cancer Research Campaign Laboratories, Nottingham) discussed experiments showing that rats developing hepatomas first have free tumour antigen in their serum, and then antigen–antibody complexes. The latter are effective in "blocking" the activity of effector lymphocytes, and disappear from the circulation within 3 days of the excision of the tumour.

Inhibition of lymphocyte effector function by T suppressor lymphocytes was a major topic of discussion. Both K. Eichmann (University of Cologne) and Leonore Herzenberg (Stanford University) presented evidence that suppressor T cells act by inhibiting the function of helper T cells (cells which help B lymphocytes to make antibody). Data from A. Basten (University of Sydney) suggest, however, that under some conditions the target of suppression may be the antibody-forming cell precursor (B cell).

There were, nevertheless, close parallels between these three systems. Thus, suppressor cells seem to belong to a discrete T cell subpopulation—bearing Ia antigens, distinctive Ly antigens, and with a characteristic tissue distribution. Most strikingly, both Eichmann's and Herzenberg's work suggests that there are categories of helper T cells which only interact with a restricted B cell subset—those destined to produce a single allelic variant of one immunoglobulin subclass (Herzenberg) or a single idiotype (Eichmann). These specialised helper cells seemed to be the targets of suppression.

The general role of suppressor cells in tolerance remains unresolved. Basten presented clear evidence that although mice made tolerant with human gamma globulin developed suppressor cells, the animals remained tolerant long after the suppressors had disappeared. For the present one may conclude that, although suppressor cells are demonstrable in a number of tolerance models, they have not been shown to be obligatory either in the induction or maintenance of unresponsiveness. Rather, they may fulfil a more subtle homoeostatic function.

One session was devoted to tolerance induced by "suiciding" specific immune responses by attaching a lethal agent to an antigen, so that the complex inactivates clones of cells bearing receptors for that antigen. This conceptually attractive approach, with obvious potential therapeutic applications, has hitherto been largely restricted to animal models using highly radiolabelled antigens. Recently, however, H. Ambrosius and his collaborators at the University of Leipzig have been testing various immunosuppressive agents, in particular 6-mercaptopurine, coupled to protein antigens. Such conjugates specifically suppress anti-protein antibody synthesis in guinea pigs. D. A. L. Davies (Searle Research Laboratories, High Wycombe) is studying the effects of a phenylalanine mustard coupled to antitumour antibody on the growth of a transplantable mouse lymphoma. The results are encouraging: administration of the drug or antibody alone is ineffective, giving the drug at the same time as antibody is effective, while the covalent drug–antibody conjugate is even more effective.

The success of this meeting was undoubtedly partly due to the fact that it brought together experts in various aspects of tolerance, and enabled them to find common ground for a variety of phenomena. Most importantly, however, it also provided a unique opportunity for immunologists from the East and the West to meet and exchange ideas. ☐

A hundred years ago

AT its last sitting the French Geographical Society broached a scheme for inducing the several French Railway Companies to place at each station a map of the vicinity, with indications of the most notable historical or economical facts connected with the district. It appears that this is the universal practice on Brazilian railway lines.

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review articles

Visual experience and cortical development

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The past twelve years have been exciting for those interested in the classical question, "How much do our visual capacities depend on innate developmental factors, how much on the moulding effects of visual experience?" Recent papers[1–4] have settled some issues, but may start new waves of controversy, and this is the occasion for writing this review.

THE oldest source of knowledge on visual development is clinical, for ophthalmologists know from their experience with amblyopia that one eye can become almost completely functionless from lack of use. This most commonly occurs when the two eyes interfere with each other, either because of strabismus (squint), or because one eye has a much greater refractive error than the other, and it can be partially prevented and reversed, at least in its early stages, by patching the good eye, thus forcing the use of the "lazy" eye that is threatening to become amblyopic. In 1963, Wiesel and Hubel[5] reported similar effects produced in cats by interfering with the normal binocular use of their eyes in the first month or two after they opened, and they discovered that the underlying change occurs in the cells of the primary visual cortex (area 17). In normal animals a high proportion of these neurones receive connections from both eyes[6], but if one eye is covered soon after birth, it makes connections with very few cortical neurones, almost all of which are then found to be connected solely to the eye which has been in continual use[5]. Furthermore, if a kitten is made strabismic by sectioning an eye muscle, or if joint usage of the eyes is prevented by covering each of them alternately, their neurones are found to receive input from one eye or the other, but very few receive input from both[7].

In their experiments Hubel and Wiesel found that covering both eyes had comparatively little effect on the cells of the cortex, suggesting a competitive element in the causation of the changes from monocular deprivation[8]. This was confirmed by Sherman, Guillery, Kaas and Sanderson[9] who made retinal lesions in one eye and closed the other. They found reduced effects of deprivation in the parts of the visual field that had no competing representation in the open eye. Hubel and Wiesel[10] found cells in the cortex of very young kittens, before the age of eye opening, that were connected to both eyes and appeared normal in other properties, and they reproduced many of these results in monkeys[11]. The apparent normality of cells in very young visually inexperienced animals, and also in binocularly deprived ones, led them to believe that the functional pattern of connections that underlies normal vision are laid down by genetically determined developmental processes. These can be interfered with or disrupted by abnormal visual experience in the "sensitive period" of the first few months of life[12], but they did not think that normal visual experience had any constructive effect in setting up the neural apparatus in the primary visual cortex.

These very important experiments with their suggested answer to the ancient dispute about the role of experience have naturally aroused great interest, and some controversy has arisen about both the facts and their interpretation. By now most of the facts discovered by Hubel and Wiesel have been confirmed by others, but there have been further advances that will be considered under four headings: ocular dominance and binocularity; specificity without experience; disparity selectivity; and the degree of modifiability of cortical neurones.

Ocular dominance and binocularity

The effects on the monocular and binocular connections of cortical neurones have proved reliably repeatable, but interesting additional knowledge has been gained by the technique of reversed suture[13–15], in which the originally closed eye is opened and the originally open eye is closed. It seems that an eye that has become disconnected can be persuaded to reconnect by renewed visual experience, provided that this occurs during the "sensitive period" and provided that the newly opened eye does not have to compete with an already connected eye that is in continued use. Thus experience can certainly have a reconstructive effect, and it is particularly interesting that the reconstructed connections are different in several ways from the previous connections that had been disrupted by the period of closure. The most striking evidence for this is provided by neurones that are in the process of being taken over by the newly opened eye but can still be driven by the other, previously open, eye. Some of these are quite unlike any cells found in normal cortex in having widely divergent preferred orientations for the two eyes, the difference sometimes being more than 70°, which is never found in neurones of normal cats and kittens More information about this is obtained by examining the properties of the series of neurones encountered while advancing an electrode through the cortex, for the newly formed connections seem to develop a sequential pattern of orientational selectivity that is similar to the old pattern, but independent and often out of step with it[16].

The effects of deprivation are certainly easier to show than are those of experience, and a nice example of this was given by Olson and Freeman[17]. They found that 2–5 d of monocular vision had a profound effect on the ocular dominance histogram, but unexpectedly, it made no difference whether the animals had been reared in total darkness or in ordinary light before the monocular experience. For the deprived eye, several weeks of ordinary vision were undone in a few days of deprivation. Following reversed suture, however, one can see that experience encourages reconstruction, and one must suspect that normal joint usage of the two eyes may have some positive effect in establishing the normal orderly matching of the connections from the two eyes.

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Specificity without visual experience

The properties of neurones in the adult primary visual cortex vary greatly, but it can be said of the vast majority that they selectively respond to only a very small proportion of the vast gamut of possible visual stimuli[6]. They are selective according to (1) the position of the stimulus, (2) whether lighter or darker than the background, (3) its direction and velocity of movement, (4) its size and orientation, and (5) the disparity or relative positioning of the image in the two eyes[18,19]. All are agreed that neurones with selectivity for (1), (2) and (3) occur in very young kittens without visual experience. Hubel and Wiesel also said there were many cells with the normal selectivity for the orientation of an extended object, as well as normal binocular connections, and it was on this basis that they concluded that all selectivity was innately determined. Barlow and Pettigrew[20] and Pettigrew[21] found clear-cut selectivity for the direction of movement of a stimulus in animals with no visual experience, and thought that the apparent orientational selectivity could be attributed to this directional selectivity. This seems to be wrong, for although many of the cells in very young animals are abnormal in their selective properties, there are about 25% (refs 2, 4) with the orientational properties of adult simple cells

With regard to older, totally deprived, kittens Hubel and Wiesel again reported many normally selective cells, whereas Barlow and Pettigrew found a remarkable lack of them, and in this respect later work has supported them[2,4,22]. Even without the evidence from older kittens, however, it is now clear that all the types of selectivity of area 17 neurones, except that for binocular disparity, occur with no visual experience. The question whether they have as high a degree of selectivity as adults is still open, and there does seem to be a possible role of experience in "tuning up" their selectvities. This is a point of some interest because of the slow attainment of the high acuity characteristic of adults (*vide infra*).

Disparity selectivity

Most studies have followed Hubel and Wiesel in representing the binocular–monocular properties of cells in the visual cortex by "ocular dominance histograms". Cells are classified into seven categories varying from those excitable only through the contralateral eye (group I), through those excitable equally well by either eye (group IV), to those excitable only by the ipsilateral eye (group VII). Such histograms tell one the relative strengths of the excitatory inputs from each eye, but they tell one nothing about the way the inputs from the two eyes interact This is important, not only because of possible facilitation, summation, or occlusion, but also because the input from an eye that does not excite a neurone can nevertheless inhibit it[21]. Thus the fact that ocular dominance histograms of very young kittens resemble those of normal adults does not mean that these animals handle binocular inputs normally. Pettigrew[21] studied this aspect of the problem in developing cortex and found a marked increase in the degree of selectivity for disparity with age and visual experience. Binocular interactions of young or inexperienced neurones occurred, but were much less precise and lacked the inhibitory component prominent in older and experienced neurones. The implication is that very young and totally deprived animals have normal ocular dominance histograms, but the critical inhibitory interactions of adults develop only with experience. This deserves repetition, especially in view of the contradictions about orientational selectivity, for it is again strong evidence for a constructive role of experience in forming the connections which combine the inputs from the two eyes.

Modifiability of other properties

A controversial problem is whether properties of the population of cortical neurones other than binocular connectivity can be affected by experience. Hirsch and Spinelli[24,25] and Blakemore and Cooper[26] reported dramatic results induced by exposing kittens to striped patterns, and Shlaer[27] produced some evidence that a prism in front of one eye deviates the receptive field positions of that eye These results were extended in later reports[28,29] and since then modifications have been described as a result of rearing animals in stroboscopic light[30,31], in an evironment of moving stripes[32–35] and in a visual environment of dots without any stripes or lines[36,37]. Some of these treatments are claimed not just to modify the proportions of neurones of different types, but also to produce neurones with properties totally unlike any that are found in normal cortex. For instance Hirsch and Spinelli[25] reported units that seemed to have a receptive field exactly matching several bars of the grating the eye had been exposed to, and a close matching of receptive field to stimulus also seems to occur when kittens are raised in an environment of dots without lines[36,37]. The cortical units reported for these kittens would certainly be most unusual in normally reared animals.

Another example of abnormal units was described by Pettigrew, Olson and Hirsch[38] in the cortex of cats reared with one eye viewing vertical, the other horizontal, stripes, for they found units, like those described by Blakemore and Van Sluyters[14] following reversed suture, that had widely divergent preferred orientations in the two eyes. Again, when one eye is rotated surgically but not closed in young kittens[39], the position of the receptive fields at different points in the cortex stay unchanged relative to the retina. The orientations are not however normal: after correction for rotation the preferred orientations in the two eyes may be widely divergent in the few remaining binocular neurones. Once more we have evidence that coordinated binocular stimulation is needed to obtain coordinated developement of the appropriate selectivities.

Behavioural consequences of selective visual diets have also been described[40,41], though these are rather subtle and hard to observe compared with the effects of monocular deprivation[42,43] There have been no reports of animals or humans with supernormal visual capacities following abnormal early visual experience, though this might be expected to arise according to some views. Some failures to obtain modification have been reported but hitherto these could be explained by differences of species, as when rabbits were used[44,45], or procedure, as when Maffei and Fiorentini[46] used vertical stripes of rather limited vertical extent and exactly regular periodicity.

At this point it seemed that, although most forms of selectivity were detectable in inexperienced cortex, they were also modifiable by subsequent visual experience. The report from Stryker and Sherk[1] however, has reopened the whole issue, for they made a careful and thorough attempt to reproduce the stripe-rearing experiment, following the procedures of Blakemore and Cooper, but they were unable to obtain significant results. The main points of difference were (1) an improved method of selecting the cells to be recorded from, aimed at obtaining more representative samples; (2) the use of a computer to control the stimulus and record the results, instead of hand-moved targets and subjective impressions, (3) ensuring that the experiment was done "blind", without the experimenters knowing the rearing conditions. Many people are likely to conclude that experimenter bias, reliance on subjective impressions, and an effectively very small sample of cells explain the dramatic findings of the previous experimenters, though it is not clear whether Stryker and Sherk hold this opinion. Against this conclusion is a brief study by Pettigrew, Olson and Hirsch[34] that was done blind, with computer control, and with positive results. There are further results, as yet unpublished, from Pettigrew's laboratory, and some of the papers already quoted contain incidental confirmation of the stripe-rearing effect (see also the note added in proof to this article). Obviously one must consider Maffei's and Fiorentini's[46] negative results again, but it seems to me unlikely that so many reasonably careful people should have been fooled so much of the time, and the clinical evidence to be described below establishes that orientational properties can be modified. It is therefore worth considering other possible explanations for the negative results.

The condition of the kitten when viewing the stripes is the natural point to probe. Was it alert? How much time did it spend looking up or down, and thus deviating the lines from their intended horizontal or vertical? What else did it see? Was it hungry, interested, rewarded? All these factors are hard to control, but may be expected to influence the results if the modification is anything like those occurring during normal learning As a possible physiological explanation it is worth pointing out that Otten and Thoenen[47], studying the effect of preganglionic stimulation in inducing enzyme formation in sympathetic ganglia, had at one point great difficulty in getting reproducible results. This was traced to the glucocorticoid level in the blood at the time of induction: if the experiment was done in the morning, when the level was low, induction failed Perhaps Stryker's and Sherk's cortical neurones ignored the stripes for the apparently irrelevant reason that they were exposed at the wrong time of day Another possibility is that the sensitive period for orientational lability is earlier than that for ocular dominance, as Daw and Wyatt suggest for modifiability of directional selectivity[35] There is also some evidence that dominance changes are slower than changes of orientation[29,48]. At all events the matter is unlikely to be accepted as closed and one must hope for more conclusive experiments in the future.

Evidence from psychophysics

These contradictory results go some way towards justifying the remark of one of the founders of cortical neurophysiology: "Recording from single units gives interesting results, but they are usually wrong". He was probably referring to other people's results, but he generously went on to say, "Visual psychophysics is dull but usually right". Even though psychophysicists do not necessarily share this confidence, it is worth trying to relate the neurophysiology to measurable psychophysical function, for there is new evidence that bears on the question of cortical modifiability. This actually lends quite powerful support to the single-unit results which have been called into question.

The main factor that limits the resolution of the adult visual system, as measured on straightforward tasks such as the visibility of a grating, is the finite size and separation of the elements of the retina. In the fovea of man this limit occurs at about 50 cycles per degree, and is set by the spacing of the receptor cells[49] Away from the fovea, the minimum resolvable angle rises with eccentricity at the rate of nearly 0.5 min per degree, so that at 10° eccentricity only about 10 cycles per degree can be resolved. The limit here is probably also set by the retinal grain, in this case traceable not to receptors but to the separation of the ganglion cells that transmit details of the image centrally. In the cat it seems to be the size of the ganglion cells' receptive fields and their separation that limit acuity, even in the area centralis[50–52].

Clearly, if the fibres connecting eye to brain made very diffuse or scrambled connections one would not be able to resolve the image to a dimension set by the separation of their pick-up zones in the retinal image. These fibres must, therefore, make connections in the adult that are in some sense appropriate and orderly.

It is interesting that, for some tasks, the full resolution available from the retinal mosaic seems not to be used. Andrews, Webb and Miller[53] investigated the accuracy with which length comparisons could be made when the markers to indicate the lengths lay up to 5° from the fovea After allowances have been made for eccentricity, the grain size that would be sufficient to enable this task to be performed was about 10 times larger, measured linearly, than that required for grating resolution. They made the interesting suggestion that this coarser mosaic is determined, not in the retina, but in the primary visual cortex, where it could correspond to the dimension, projected into the visual field, of the repeating subunit called a "hypercolumn" by Hubel and Wiesel[54,55]. This is the area of cortex, about 1 or 2 mm square, within which a complete cycle of ocular dominance is contained when moving across the cortex in one direction, and a complete cycle of orientational selectivity when moving in the orthogonal direction. It is thus a unit cell containing all orientation selectivities and all degrees of ocular dominance. Although the receptive fields of successive units isolated on moving an electrode across a hypercolumn show an average tendency to move across the visual field in the manner predicted from the overall mapping, an exact progression is not found[55,56]; the units are scattered around the expected positions in an apparently random manner, so it seems that the topographical orderliness for retinal position has been replaced by orderliness for orientational selectivity and ocular dominance On the hypothesis being advanced, the output from a hypercolumn carries the information as to which hypercolumn is involved, but no information about position within it, and the consequent discarding of information causes the loss of resolution for the type of task Andrews *et al.*[53] studied.

Another task that suggests massive reduction of positional information is the counting of narrowly spaced lines that has been studied recently by Atkinson *et al.*[57]. They found that subjects started to make serious errors in counting even small numbers of lines when these are crowded close together: possibly the output from a hypercolumn can only signal "one" or "many", and for exact counting the lines in the target must fall on separate hypercolumns.

Thus, some tasks seem to be limited by the retinal grain, others by a coarser grain that may correspond to the hypercolumnar structure of the primary visual cortex. There are yet other tasks which at first seem to demand an even greater degree of precision than grating acuity. These are tasks like detecting the displacement of a vernier, the curvature of a line, or comparing the disparity of a pair of lines, and their common feature is that they require the detection of the relative positions of features in the stimulus that lie close to each other in the visual field. Since they are close, they could be within the area of visual field covered by one hypercolumn, and this type of information about relative position is not discarded, but jealously preserved. The precision with which these tasks can be performed is extremely high, of the order of a few seconds of arc, but Andrews, Butcher and Buckley[58] show that this is no higher than the accuracy expected from efficient use of the information in an image limited by the retinal grain The improvement results from averaging, and simply corresponds to the fact that a mean is more precise than the individual measurements on which it is based. Thus there is no mystery about how the information required for the task gets to the brain, but a highly precise and orderly arrangement does seem to be required in order to extract it, and this is presumably one of the tasks of the hypercolumns in the primary visual cortex.

To summarise, then, psychophysics suggests there must be precisely ordered connections from eye to brain to preserve details down to the size of the retinal mosaic, and an even more precisely ordered arrangement within V1 for extracting the relative mean positions of features present in the image. But there is nothing impossible or implausible in these requirements, for hypercolumns are 1 or 2 mm across and contain some 10^5 cells, which allows plenty of scope for the operations demanded. The next step could be a reduction of positional information in the visual message brought about by retaining information about local pattern or relative position, but discarding absolute positional information, thus simplifying the picture by coarsening the mosaic to that of the hypercolumnar structure of V1.

Precise, orderly, and appropriate functional neurone–neurone connections are the essence of these operations. If they are made once and for all and are not modified or varied in potency by visual experience one would have a visual system whose performance depended exclusively upon its innate connectivity, a model sometimes referred to as the "hard-wired" cortex. A hardline advocate of hardwiring will explain effects of experience exclusively in terms of disruption of innate connections, but arguments can be made on general grounds for a more constructive role. Platt[59] for instance held that a precision of a few seconds of arc could not possibly be laid down under genetic instruction, and would be hopelessly inflexible even if it could be. Andrews[60] argues that experience must be important in determining the metrical properties of perception, and Blakemore and Van Sluyters[2] suggest that experience ensures the alignment of the contributions from each eye to binocular receptive fields. Although these ideas are interesting they are not by themselves fully convincing. A teleological argument from the very existence of the sensitive period carries some weight, for it really is hard to believe in the survival value of a period of pure disruptibility, whereas a period of refinement and adjustment by experience would make good sense. At the moment there are no hard facts establishing such a role, but new evidence shows where the case for hardwiring is incomplete, and hints where visual experience may have its effects. Furthermore, recent experiments on the transfer of after effects support some of the concepts relating to cortical neurophysiology and psychophysics that have been discussed in this article.

Development of visual acuity

The neurophysiological argument for an innately connected cortex rests on the presence of selectively sensitive neurones in young, visually inexperienced kittens and monkeys One could rule out any role of experience if such animals had full adult acuity, but this is far from the case. Acuity develops rather slowly in kittens[61,62], and does not reach its full adult value until quite late in the sensitive period. This could be a matter of removing other impediments to resolution, and as in all behavioural experiments one cannot be sure that the kitten is making full use of his sensory mechanisms, but the behavioural result is here supplemented by the study of cortical evoked potentials[61]. In humans too it seems that acuity is poor in young infants[63,64] and reaches a value comparable with that of the adult long after the system seems to be optically efficient

One would like to know more about the effects of early binocular deprivation on visual capacities after sight is restored, for clearly if full acuity can ever be found in an animal that has been totally vision-deprived up to the moment of testing, this would rule out any important role for experience in performing resolution tasks. Clinical evidence on this is not very conclusive[65–67] and results on animals are not yet very extensive[43,61,62,67] but it does not seem that full visual capacities ever mature without considerable experience, and they often fail to do so if the experience occurs after infancy. If full performance cannot be achieved without experience, the implication is that normal experience aids cortical development and does not merely fail to hinder it.

Meridional amblyopia

The starting point of this whole enquiry was the fact that an eye can become blind when it is in competition with the other, functionally superior, eye, and the discovery that a similar condition occurs in cats and results from changes in the connections of cells in the primary visual cortex It has long been recognised clinically that individuals with severe, long standing, astigmatism often fail to achieve good resolution in the affected meridian, even with the best correction of the optical condition. Testing the acuity of such subjects by generating interference patterns directly on their retinae has ruled out the possibility of a residual optical defect, and has now shown that this "meridional amblyopia", as it is called, is really of neurological origin[69]. Kittens reared wearing goggles containing cylindrical lenses have been shown to lack cortical neurones tuned to the blurred meridian, thus completing the cycle of evidence[70].

In spite of the difficulties in repeating the original stripe-rearing experiments, this clinical condition seems to establish beyond any reasonable doubt that the orientational properties of cortical neurones can be influenced by experience, though it does not of course establish that the changes are as described in the original neurophysiological experiments

Stereo blindness

Strong neurophysiological evidence for a positive role of visual experience in setting up the visual cortex was given by Pettigrew's finding that cells narrowly selective for a particular disparity are not found in kittens that have had no binocular visual experience The high incidence of anomalies of stereoscopic vision is therefore intriguing. Richards[71] estimates that 14% of the population lack the full ability to use binocular disparities to judge depths, and he reports interesting evidence that the stereo blind may fall into at least two classes—those who cannot use the convergent disparities of objects closer than the fixation point, and those who cannot use the divergent disparities of objects lying further away than the fixation point. Now phorias, or tendencies to strabismus, are common in infancy, and could cause deprivation of binocular stimulation. If the phoria is of the convergent variety, objects at the attempted fixation distance will have divergent disparities, but the system will tend to be starved of convergent disparities. Similarly those with a divergent phoria will be exposed to convergent disparities but will tend to be deprived of exposure to divergent disparities. The two forms of stereo blindness could thus be other examples of deprivation amblyopia

Recent clinical evidence suggests that a week of monocular deprivation in infancy can lead to unilateral amblyopia in later life[72,73], so the sensitivity to deprivation is certainly very high in humans. The more subtle role of phorias in causing stereo blindness seems to be another potentially fertile field for correlating neurophysiological and clinical results.

Transfer of after effects

The view that proper stereopsis depends upon experience is supported by recent experiments comparing the transfer of after effects in normal adults and those who have seriously impaired stereoscopic vision, but have good monocular vision in both eyes, and no diplopia. From the cortical neurophysiology one might hazard a guess that they do not have disparity selective cells in area 17, and probably possess a population of neurones like that found after strabismus or alternating occlusion in kittens: that is, many cells monocularly dominated from each eye, but very few that receive binocular connections. Now there are a number of illusions such as motion after effects, tilt after effects, and distortions in the appearance and threshold modulation of gratings, that are commonly attributed to fatigue of cortical neurones; Maffei, Fiorentini, and Bisti[74] have recently shown that such fatigue does occur. Furthermore, such illusions normally transfer from one eye to the other, so that if the fatiguing stimulus is given to one eye, the illusion can be detected when that eye is closed and the other eye opened. Now if stereoblind individuals lack binocular neurones, such transfer should be impossible, and that is precisely the result that has been found[75–78]. In an ingenious application of this finding Hohman and Creutzfeldt[79] have concluded that the sensitive or labile period for development of binocular neurones in humans extends to the third year.

It is interesting that Ganz, Hirsch and Tieman[80] found normal interocular transfer of learned pattern discrimination in cats reared with alternating occlusion of the two eyes and therefore presumably possessing very few binocular cells in their primary visual cortex. This result does not conflict with the absence of transfer of after effects in such a cortex, because the tasks performed are so different. The persistence of transfer of learned discriminations presumably means that they were performed at a level where information as to which eye provided the image had been discarded.

Conclusions

The neurophysiological experiments that have helped so much in understanding the normal and abnormal development of the visual cortex are difficult and arduous to perform. The preparation involves several hours of surgery, and this is followed by a prolonged period of recording during which the animal has to be maintained lightly anaesthetised and in excellent condition. Worthwhile results are rarely obtained in less than 16 h, and recording often continues round the clock for 48 h or more. On top of this there are the frustrations and difficulties of maintaining cats in colonies that are notoriously liable to obliteration by epidemics. In view of this it is not the controversy but the agreement with Hubel and Wiesel's results that is surprising, and we owe to them most of our knowledge of the visual cortex as a precisely arranged structure laid down before visual experience, and thus innate. A good deal has, however, been added to their picture, and this shows it is not really established that all is innate. First, neither an inexperienced animal, nor an inexperienced neurone, has been shown to have the normal powers of resolution, and selectivity for disparity especially seems to require experience. Second, there is the evidence that experience after monocular deprivation causes the construction of a differently organised cortex. Third, Cragg[3] has recently published evidence showing that less than 1% of synapses in primary visual cortex develop before eye-opening, and hence there is, if Hubel and Wiesel are correct, an embarrassing difficulty in explaining what the other 99% do! But this merely underlines the disquieting fact that we do not fully understand what part area 17 plays in vision—itself another reason for caution in making pronouncements upon the relative importance of innate and experiential factors in creating the intricate mechanisms that underlie our visual capacities.

There is so much to be explained in the formation and adjustment of the connections between the 10^9 or so cells of our visual cortex that it is perhaps time to abandon the idea that nature and nurture are rivals at this task. That is too simple a view; instead one should suspect that they work in collusion, and seek how and where this may occur. Does the nervous activity from experience influence the survival and effectiveness of excitatory synapses? Does it exert its effect on the pattern of connections of an intrinsic inhibitory system that is established after the innate excitatory one? Or is there an interaction between nervous activity and the development process itself, as seems to occur in goldfish tectum[81]? The evidence reviewed here suggests that some of the answers are still waiting to be found in the mechanisms of the primary visual cortex that analyse details of the image and combine the information from the two eyes.

Note added in proof: Three new twists must now be added to the story on the effects of selective exposure First, Hirsch and Levental[82] report more experiments in which kittens were reared wearing goggles of the type used in the original experiments of Hirsch and Spinelli[24,25] In their new experiments the eyes viewed stripes within the goggles that were either left oblique for one eye and right oblique for the other, or horizontal for one eye and vertical for the other as in the original experiment For the latter condition they confirmed, using more conventional neurophysiological techniques, that cells selective for horizontal orientations were almost all connected only to the eye that had viewed horizontal stripes, and similarly for the vertically orientated cells. There were a few cells which broke this rule by responding to verticals through the horizontally exposed eye and vice versa, but there were no cells at all that responded to oblique lines. The animals reared viewing obliques had many obliquely sensitive cells connected to the appropriate eye but also had many cells which responded to horizontal or vertical stripes through each eye They conclude that genetic factors are sufficient to develop horizontal and vertical orientational selectivity, but that experience is required for the obliques.

The second twist is a note added to Stryker's and Sherk's paper[1] confirming these results for kittens reared with the horizontal/vertical goggles, using their more precise quantitative techniques. It is not yet known whether the differences between the results using goggles, and those from Blakemore's and Cooper's cylinders, is due to more effective control of the visual experience, or whether the conflicting experience of the two eyes in the goggles is the critical factor.

Finally, Grobstein and Chow[83] have reviewed their own work on the development of cortical specificity in the rabbit, and have also covered much of the material in this article. Their general conclusion is that individual sensory experience is a creative factor in establishing the functional organisation of the mammalian visual system, though they believe it affects the rate of development more than the end state reached.

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articles

New evidence regarding the Quaternary geology, archaeology and hominids of Chesowanja, Kenya

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Deposits yielding australopithecines, other fossil mammals and an industry recalling the Oldowan are succeeded by an Acheulian assemblage, overlain by flood plain silts with abundant spreads of later artifacts. The structure is dominated by an asymmetric anticline. Palaeoenvironments are reconstructed and the nature of the raw materials and of the tool makers themselves are briefly discussed.

CHESOWANJA is a Pleistocene fossil and artifact locality in the Northern Rift Valley of Kenya, about 1.5 km west of the track between Mukutan and Tangulbei, at 36° 12′ E, 0° 39′ N, and 8 km WNW of the point at which the Mukutan river gorge cuts through the Laikipia Escarpment. A diverse fauna includes the partial cranium of a robust australopithecine[1,2].

We report here results from more detailed geological mapping in 1973 and 1974. A remarkable sequence of artifact-bearing levels has been discovered together with a further hominid. The original geological interpretation has been shown to be in error, necessitating re-assessments of the local stratigraphic relationships, the palaeoenvironmental setting and the age of the sequence.

The area is important because the succession of rich artifact assemblages, from a relatively small and well defined region, spans a period probably well in excess of a million years. The associated geology and fossil fauna enable palaeoenvironmental contexts to be inferred for the various phases of hominid activity.

Geological succession

The local sequence of strata (Fig. 1) comprises four lithological units: the Chemoigut Formation, Chesowanja Formation, Karau Formation and Mukutan Beds. Formal descriptions of these and of the geology of the surrounding area will be published elsewhere.

The Chemoigut Formation consists of a sequence of silts and clays with intercalated horizons of coarse tuffaceous and pumiceous sandstone and fine conglomerate. There are calcareous bands and concretions, some of which are of algal origin, and thin flaggy and nodular calcrete horizons, several containing abundant root casts. There is also evidence of channelling. The observed thickness of the unit is ~ 25–30 m. The succession overlies red clays containing nodular calcrete, which elsewhere rest on very weathered ankaramitic basalts.

Artifacts have been found eroded out at a number of different sites from at least five horizons. Australopithecine remains come from the two highest levels. The fauna is listed in Table 1.

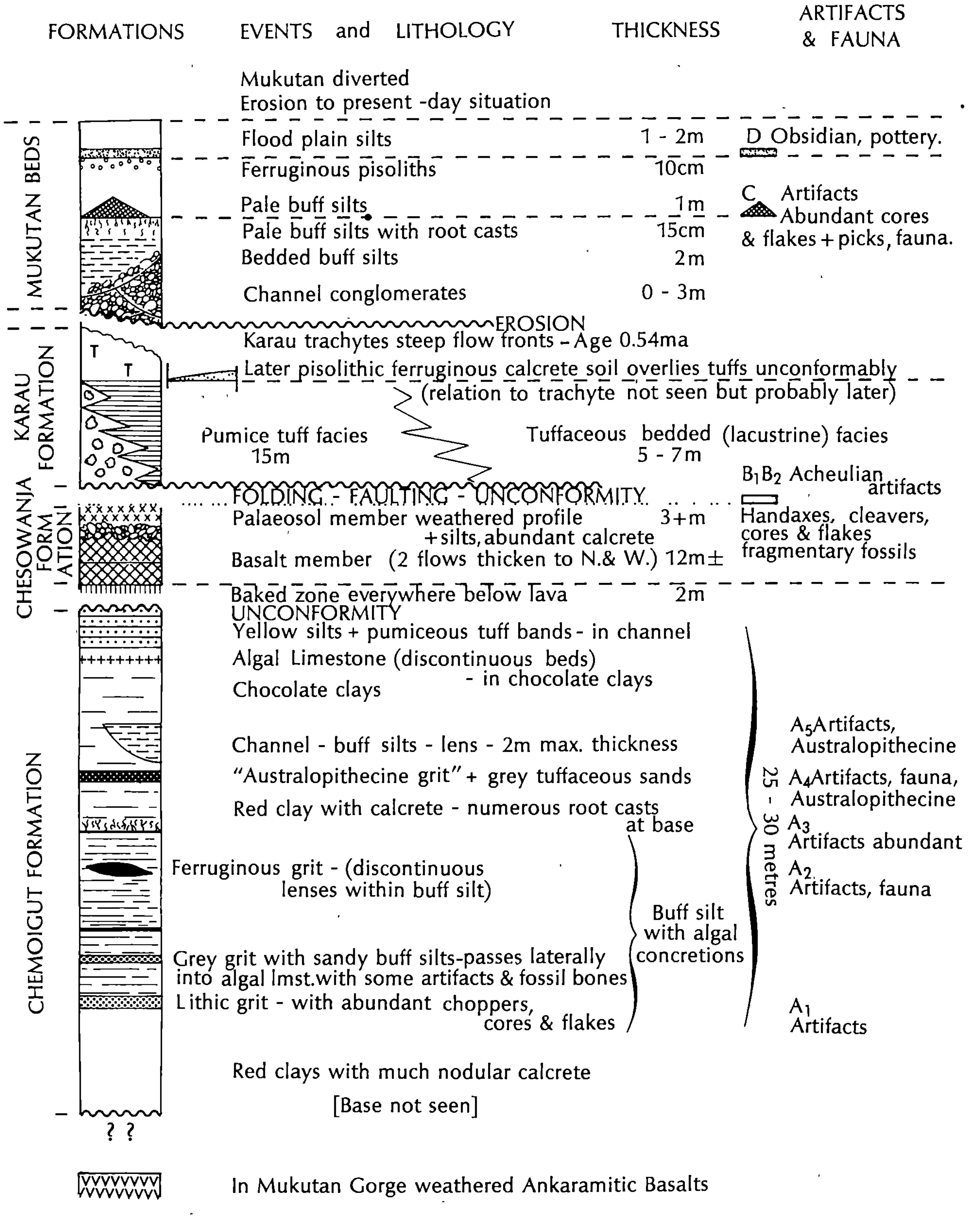

FORMATIONS
EVENTS and LITHOLOGY
THICKNESS
ARTIFACTS & FAUNA
Mukutan diverted
Erosion to present -day situation
MUKUTAN BEDS
Flood plain silts 1 - 2m
D Obsidian, pottery.
Ferruginous pisoliths 10cm
Pale buff silts 1m
C Artifacts
Abundant cores & flakes + picks, fauna.
Pale buff silts with root casts 15cm
Bedded buff silts 2m
Channel conglomerates 0 - 3m
EROSION
CHESOWANJA KARAU FORMATION
T
Karau trachytes steep flow fronts - Age 0.54ma
Later pisolithic ferruginous calcrete soil overlies tuffs unconformably
(relation to trachyte not seen but probably later)
Pumice tuff facies 15m
Tuffaceous bedded (lacustrine) facies 5 - 7m
FOLDING - FAULTING - UNCONFORMITY
B_1B_2 Acheulian artifacts
Handaxes, cleavers, cores & flakes fragmentary fossils
CHESOWANJA FORMATION
Palaeosol member weathered profile + silts, abundant calcrete 3+m
Basalt member (2 flows thicken to N.& W.) 12m±
Baked zone everywhere below lava 2m
UNCONFORMITY
CHEMOIGUT FORMATION
Yellow silts + pumiceous tuff bands - in channel
Algal Limestone (discontinuous beds) - in chocolate clays
Chocolate clays
Channel - buff silts - lens - 2m max. thickness
"Australopithecine grit" + grey tuffaceous sands
Red clay with calcrete - numerous root casts at base
Ferruginous grit - (discontinuous lenses within buff silt)
Buff silt with algal concretions
Grey grit with sandy buff silts-passes laterally into algal lmst.with some artifacts & fossil bones
Lithic grit - with abundant choppers, cores & flakes
25 - 30 metres
A_5 Artifacts, Australopithecine
A_4 Artifacts, fauna, Australopithecine
A_3 Artifacts abundant
A_2 Artifacts, fauna
A_1 Artifacts
Red clays with much nodular calcrete
[Base not seen]
? ?
In Mukutan Gorge weathered Ankaramitic Basalts

Fig. 1 Sequence of strata exposed in the Chesowanja area

The Chesowanja Formation includes a basalt member composed of two flows, the lower of which is more fissile, and has baked and reddened the upper 2 m of the underlying Chemoigut Formation. It varies from 5 to 10 m in thickness. The upper flow is about 6 m thick. The surface of the upper flow is deeply weathered and large core stones of basalt occur at the base of a palaeosol complex 3 metre thick (palaeosol member, Fig. 1) containing abundant calcrete concretions. An assemblage of Acheulian artifacts with a sparse but persistent accompaniment of fragmentary fossil material occurs at the upper surface of the formation.

In the west, the lower sediments of the Karau Formation are coarse pumice tuffs about 15 m thick, which lie unconformably over the Chesowanja Formation. The pumice tuffs can be equated with 5 to 7 m of fine tuffaceous, well bedded sediments in the east that show graded and current bedding. These seem to represent a penecontemporaneous lacustrine facies of the pumice tuffs. The upper member is the Karau trachyte which exhibits steep flow fronts to the west of the main fossil and artifact localities.

The Karau Formation lies under local unconformities in the vicinity of the artifact and faunal areas; elsewhere it lies under a thin but persistent weathered horizon which has a patchy distribution and is characterised by ferruginous pisoliths. (see Fig. 1).

In the eastern part of the area the Karau formation is overlain by Mukutan Beds, a laterally extensive succession of channel conglomerates and silts approximately 8 m thick. The best exposures are in the Losokweta river valley and along the sides of the abandoned channel of the Mukutan River. Artifacts and fauna occur abundantly along this former course of the Mukutan.

Geometry of the units

The local relationship between the lithological units is shown in Fig. 2. The Chemoigut and Chesowanja Formations are folded into an asymmetric anticline, the axis of which trends north–south. The inclination of the beds on the western limb is about 11°. On the east they are inclined more steeply, and dips in excess of 60° are typical. The characteristically spheroidal weathered top of the upper basalt flow has been seen at only two points.

The Chemoigut sediments are exposed within the core of the anticlinal structure in three windows, each approximately 200 m across from east to west. The three windows have a total outcrop length of about 1 km from north to south along the main axis of the anticline.

Horizontal strata of the Karau Formation rest unconformably upon the flanks of the anticline indicating post-tectonic deposition. The absence of *in situ* material of this formation resting on Chemoigut sediments suggests that the Karau Formation was deposited before the fold structure in the basalts was breached. The upstanding form of the anticline at that time may account for the differing facies of the rocks on the east and west of the feature assigned to the tuffaceous member of the Karau Formation.

The more recent Mukutan Beds crop out patchily over an extensive area to the east of the Losokweta River, where they lie under the westerly sloping pediment and wash plain at the foot of the Laikipia Escarpment.

Another hominid

The partial cranium of a robust australopithecine (KNM-CH-1) reported previously[2], came from the unit now called the grey tuffaceous grit within the Chemoigut Formation. More hominid fragments were found in 1973, belonging to another individual (KNM-CH-302). They came from within chocolate clays of the same formation (see Fig. 1) some 3 m higher in the sequence than the original cranial fragment. Although the fragments are surface finds, the local topographic situation (an isolated clay spur yielding fresh artifacts) suggests that they were not far removed from their original position.

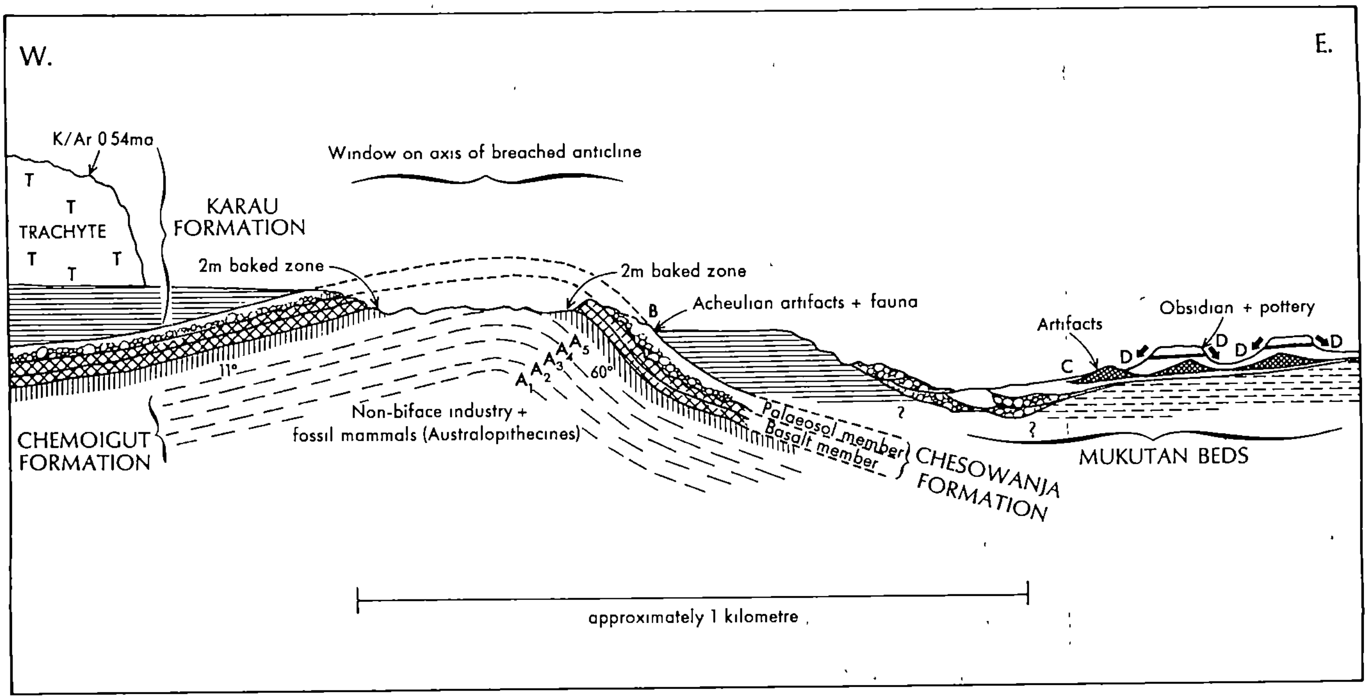
The specimens consist of a number of fragments of two molar teeth, probably of the same individual. One is a fragmentary right M^1 or M^2 crown showing features typical of the dentition of robust australopithecines. From the material available it is impossible to ascertain the exact dimensions of the tooth.

Archaeology

Archaeological material comes from at least eight levels within the vertical sequence of some 50 m of sediment and lava already described. All the occurrences are within about 1 km of each other. From their stratigraphic setting the artifacts can be assigned to four age groups, indicated as A_1, A_2, and so on, B, C, and D (Figs 1 and 2).

Group A. These assemblages of cores, chopping tools and flakes without bifaces and cleavers are confined to the Chemoigut Formation. They occur on at least five levels, including those from which the hominid specimens and other mammalian fossils also come. The five artifact-bearing levels have at least ten rich sites from which associations of artifacts are being eroded. No excavations have yet been undertaken but small samples from four of the sites have been collected. The 220 pieces include: 17 choppers; 8 polyhedrons; 4 discoids; 3

Fig. 2 Diagrammatic section across the Chesowanja area showing relationships between lithological units (not to scale). (We thank Dr Mary Leakey for the comment that the 'non-biface industry' may prove to be Developed Oldowan.)



protobifaces; 6 scrapers; 5 sundry tools; and 177 samples of waste, debitage, flakes, and so on. In spite of a detailed search no obvious hand axes or cleavers were found within the Chemoigut Formation windows, although they are abundant in group B.

Group B. An Acheulian assemblage with bifaces, cleavers, cores and flakes, comes from the surface of the palaeosol member. The latter is a complex weathering profile developed on the upper flow of the basalt member of the Chesowanja Formation, on the eastern limb of the anticline. At another locality on the same horizon small flakes and cores are dominant. A rather different, younger assemblage of artifacts occurs in association with the weathered horizon containing ferruginous pisoliths.

Group C. A third group of artifacts comprises mainly large cores with piles and spreads of associated flakes, together with occasional bifaces showing an economy of flaking technique, double ended picks, and beaked implements recalling the type 'Sangoan' in morphology[3,4]. Very large local accumulations of this material are found at numerous tool sites along the dry river bed marking the former course of the Mukutan. They are being re-excavated from the pale buff silts comprising the former flood plain of the Mukutan by modern stream action and sheet erosion.

Group D. Obsidian flakes and pottery occur intermingled with debris of group C. They seem to be eroding out from an horizon characteristerised by the presence of ferruginous pisoliths, stratigraphically a little above the level of assemblage C within the Mukutan Beds.

More recently, the river Mukutan has been diverted for irrigation purposes. Wood from a burnt stump of a large tree along the abandoned channel has yielded a radiocarbon date of 230 ± 110 yr b.p. (unpublished, Birmingham University Radiocarbon Laboratory Date BIRM 537).

Age

In the original publication[2] and in later comments[5,6] it was suggested that the sediments now placed in the Chemoigut Formation were probably younger than 1.2 Myr, but it was stressed that this estimate depended for its accuracy on the geological interpretation. Subsequent work has shown that the relationship of the Chepchuk trachyte (from which the 1.2 Myr date was obtained) to the fossiliferous sediments is not yet established.

The fauna from the Chemoigut Formation (Table 1) is not inconsistent with the original assessment. The *Deinotherium bozasi* Arambourg from the formation would, however, be the youngest known if the K–Ar age of less than 1.2 Myr were accepted. This may prove to be the case, but until additional isotopic dates are available the faunal evidence suggests an age for the formation in excess of 1.34 Myr, which is the date of the latest *D. bozasi* recorded elsewhere (below tuff J, Shungura Formation, Omo[7]).

Palaeomagnetic measurements on material from the baked zone of the Chemoigut sediments lying immediately under the Chesowanja basalt member indicate a reversed polarity (A. Brock, personal communication) and preliminary readings suggest that other levels in the Chemoigut Formation are also reversed (P. Dagley, personal communication). Taking other evidence into consideration, this would place the age of the basalt and the underlying sediments in the Matuyama Reversed Epoch between about 0.7 and 2.4 Myr BP.

The Chesowanja Formation is overlain by the Karau Formation, and a ^{40}K–^{40}Ar age determination on the Karau trachyte has given a date of 0.54 Myr BP. Micas within the tuffaceous member of the Karau Formation in its lacustrine facies, are also potentially dateable. Preliminary palaeomagnetic measurements of the tuffaceous member have produced a number of normal readings (P. Dagley, personal communication).

Table 1 Fauna from the Chemoigut Formation

Mollusca	
Gastropoda	*Bellamya* sp.
Bivalvia	
Pisces	
Siluriformes	
Reptilia	
Chelonia	
Trionychidae	
Pelomedusidae	
Crocodilia	*Crocodylus niloticus* Laurenti
Mammalia	
Primates	
Cercopithecidae	*Simopithecus* sp. sp. indet
Hominidae	*Australopithecus* sp. (2 individuals)
Proboscidea	
Elephantidae	*Elephas* c.f. *recki* Dietrich
Deinotheriidae	*Deinotherium bozasi* Arambourg
Perissodactyla	
Equidae	*Equus* sp.
Rhinocerotidae	*Ceratotherium* sp.
Artiodactyla	
Suidae	*Mesochoerus* sp. *Metridiochoerus andrewsi* *Metridiochoerus jacksoni* probably 2 other species
Hippopotamidae	*Hippopotamus* c.f. *amphibius* Linn *Hippopotamus* sp.
Giraffidae	
Bovidae	
Tragelaphini	*Tragelaphus* sp.
? Tragelaphini	
Bovini	
Reduncini	*Kobus* sp.
Alcelaphini	? *Megalotragus kattwinkeli* Schwarz ? *Connochaetes* sp. Small species Smaller species
Antilopini	? *Antidorcas* sp.

Palaeoenvironment

The artifact assemblages of Group A have not yielded examples of hand axes or cleavers. They lie under and predate the typical Acheulian elements of group B and thus invite comparison with the 'non-biface' Oldowan Industry at Olduvai Gorge. The sediments of the Chemoigut Formation indicate an environment on the margins of a saline lake with a fluctuating water level. Several fresh cores and flakes have a calcium carbonate encrustation of algal origin. It is interesting that the Bed I and lower Bed II Oldowan assemblages of Olduvai also occur around the margins of a saline lake[8].

The Acheulian of group B is from a subaerial environment on the upper surface of a complex weathered profile. The artifact sites lay to the east of an anticlinal ridge at the foot of a pronounced basalt feature caused by the sharp flexure forming the eastern limb. A lake may have existed only a short distance further to the east as the Acheulian surface is overlain by well bedded, tuffaceous strata, indicating deposition in standing water. A sudden local rise in the water level would have occurred with the influx into the catchment area of large amounts of pumiceous ash from the trachyte centre of Karau which lies 4 km to the South-west.

The piles and spreads of artifacts in group C represent stone working sites and occur upon root cast surfaces in flood plain silts. Adjacent fresh flakes can be fitted together on to the large cores from which they were struck. The industry was based on raw material of phonolite cobbles from anastomosing water-courses of the former Mukutan river system. The artifacts were stratified beneath flood plain silts and wash deposits as the local base level rose. Local breaks in sedimentation occur. One of the most pronounced of these is marked by ferruginous pisoliths. Microlithic tools and flakes in obsidian and other lithologies, together with pottery, were stratified during this phase of flood plain deposition which culminated some 2 m above the horizon of group C. Later incision initiated a cycle of erosion which is still continuing and has produced remnant mesas (Fig. 2). This cycle has re-excavated broad channels to below the level of group C and has produced an intermixing, on the present day surface, of elements of groups C and D, together with occasional implements in iron The exact relationship of these artifacts will only be established by careful excavation of *in situ* material.

The main flow of the Mukutan river was diverted towards the north by the Njemps tribesmen 100 or more years ago to irrigate the wash plain for agriculture.

Raw materials

An ongoing study is concerned with identification of the various rock types used by makers of the tools in groups A–D. The earliest tool kits reveal great petrological variety including different trachytes, phonolites and welded tuffs, together with occasional basalts. The Acheulian raw materials of group B are restricted to trachyte with some phonolite and a few small pieces in welded tuff. Group C is almost entirely based on water rounded blocks and cobbles of phonolite.

Various volcanic rocks occur in the area, surrounding the artifact sites. Thus it is possible to carry out petrological and geochemical investigations, including trace element analyses, to identify the source rocks of the artifacts. This will permit an estimate of the minimum ranges over which the early stone technologists travelled to collect their raw materials.

The fact that two more individuals of robust australopithecines have been found from horizons yielding abundant artifacts (others are known from Olduvai Gorge and East Rudolf), poses the question: "Who made the tools?"

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Significance of autogenously regulated and constitutive synthesis of regulatory proteins in repressible biosynthetic systems

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The functional implications of the different modes of regulation have been examined systematically. The results lead to certain predictions. The regulatory protein in repressor-controlled systems is constitutively synthesised. In activator-controlled systems synthesis of the regulatory protein is autogenously regulated. There is favourable agreement between these predictions and published experimental evidence.

REGULATION of specific enzyme synthesis has been most thoroughly characterised in the inducible catabolic systems of enteric bacteria[1,2], and in the temperate bacteriophage λ (ref. 3). Regulation of enzyme synthesis in the biosynthetic systems of bacteria has remained more obscure. Among studies on systems of the latter type, those on the tryptophan biosynthetic system in *Escherichia coli* are probably most advanced. Regulation of this operon clearly involves the classical mechanism proposed by Jacob and Monod[4]. Trytophan, the end product of the pathway, is a corepressor that can combine with an aporepressor molecule to form a "repressor" that binds to the tryptophan operator and blocks transcription[5–10]. Arginine regulation in *E. coli* may also involve a classical aporepressor[11,12], although the evidence is less extensive.

In contrast to the tryptophan and arginine systems, there is no evidence of a classical aporepressor for the histidine and isoleucine–valine biosynthetic systems, although these systems also have been investigated extensively. Consequently, basic assumptions about repressor function have been re-examined critically and other mechanisms have been proposed.

Histidyl-tRNA, rather than histidine itself, has been clearly implicated in repression control of the histidine operon in *Salmonella typhimurium* (see review by Brenner and Ames[13]). Ames and his coworkers[13–15] suggested that a complex of histidyl-tRNA synthetase (aporepressor) and aminoacylated tRNA^{His} (corepressor) may be a repressor of the histidine operon In contrast to this suggestion that the synthetase is a negative element in control, Wyche *et al.*[16] reported evidence that the synthetase is a positive element. There is evidence for yet another type of model in which the first enzyme in the histidine biosynthetic pathway (N-1-[5′-phosphoribosyl] adenosine triphosphate: pyrophosphate phosphoribosyltransferase, EC 2 4.2.17) has a role in repression control. Goldberger and coworkers[17–19] proposed that the first enzyme acts as a negative element in the control. But involvement of the first enzyme as a positive element in the control of the histidine operon also has been suggested[19,20].

These and other possible models now can be tested with purified (or partially purified) components in an *in vitro* transcription system and in a coupled transcription–translation system Blasi *et al.*[21] reported that the first enzyme blocks *in vitro* transcription of the histidine operon but, surprisingly, histidyl-tRNA was not required for this result. In contrast, Artz and Broach[22] obtained evidence that a positive element is required for expression of the histidine operon in the coupled transcription–translation system. Their studies also suggest that transcription of the histidine operon requires translation of its specific mRNA. One of several explanations consistent with these observations is that translation produces a positive factor required for transcription[22]. Thus, the results so far from these *in vitro* systems are consistent with the notion that a product of the histidine operon is involved in regulating its own synthesis. In one case, however, this product is thought to be the first enzyme and a negative element in control, whereas in the other, this product is an unspecified positive factor. A combination of the two is also a possibility.

Similar developments have occurred in the study of the isoleucine–valine (*ilv*) biosynthetic system. This system involves multivalent repression[23,24] in a branched set of pathways[25] and is, therefore, more complex than the histidine system. As is the case for the histidine operon, no classical aporepressor has been identified for the isoleucine–valine system. On the basis of biochemical data Hatfield and Burns[26] suggested a model of repression control for the *ilvADE* operon in *S. typhimurium* that involves the first enzyme of the isoleucine pathway (threonine deaminase, EC 4 2.1.16), leucyl-tRNA, valine, threonine and isoleucine. According to this model, which now has been modified and extended to cover regulation of the other *ilv* operons in *E. coli* and *S. typhimurium*[27], the first enzyme is an aporepressor and the multivalent corepressors are isoleucyl-tRNA, leucyl-tRNA and valyl-tRNA. On the other hand, Levinthal *et al.*[28] have described a regulatory mutant of *E. coli* in which the expression of the *ilvADE* operon is diminished and unresponsive to variations in the branched chain amino acids. This phenotype results from a single point mutation in the structural gene for the first enzyme of the isoleucine pathway. Based on these and other genetic results, Levinthal *et al.*[28] suggested that the first enzyme is a positive element controlling expression of the isoleucine–valine system.

A defective transducing phage harbouring the *ilv* genes[29] is being used for *in vitro* transcription and coupled transcription–translation assays, to study the molecular events in the regulation of the isoleucine–valine biosynthetic system of *E. coli*.

As this brief introduction indicates, there are repressible biosynthetic operons whose regulation involves a classical, constitutively synthesised aporepressor. There also are examples of such systems that involve autogenous regulation, whereby a regulatory protein directly modulates expression of its own structural gene[30]. Although in no case has it been demonstrated that either of these is the sole mechanism of control, the question arises: what are the functional implications of these two established modes of regulation? This question cannot be answered by comparing directly two representative systems (for example, tryptophan and histidine) because there may be still other elements involved in their control and because the systems are different in many ways that are irrelevant to a comparison of the two modes of regulation *per se*. An answer requires a more controlled comparison in which the two systems are identical in every respect except for the difference in a regulatory mechanism. This is difficult to do experimentally. At present a more practical approach is to compare mathematically models that accurately represent the different types of regulation. Such comparisons have been made and the details, unnecessary here, are available[31]. Before presenting the results it will be useful to enumerate the criteria for functional effectiveness that have been used in comparing the various models.

Criteria for functional effectiveness

Just as numerous chemical and physical criteria make possible characterisation of the molecular components of biological systems, well defined criteria make possible characterisation of the functional behaviour of an integrated system. For simplicity, in the analysis that follows I deal specifically with the regulation of unbranched pathways for the biosynthesis of amino acids. The following criteria for functional effectiveness can be formulated for these systems. It should be noted that none of these criteria assumes anything about the type of regulatory mechanism involved.

(1) Minimisation of the change in the level of end product as it shifts from one steady state to another in response to a change in demand for end product. This implies that the end product will not be depleted to such an extent that its rate of utilisation is limited when the demand is increased.

(2) Responsiveness to change in the availability of initial substrate. This is essential for the redistribution of common intermediary metabolites that occurs in response to a changing environment when the available carbon is limited.

(3) Reduction of enzyme synthesis when the end product is supplied exogenously. Since the biosynthetic enzymes become superfluous when the end product is available preformed in the environment, the importance of this criterion for cellular economy is obvious.

(4) Stability. Presumably one of the prime functions of pathways synthesising amino acids is to provide a relatively constant supply of their end products for protein synthesis. An effective system would not oscillate wildly, starving the organism for end product during one phase and overproducing and wasting it in the next. The impaired growth of mutants exhibiting such instability is readily apparent[32].

(5) Temporal responsiveness to change. The regulatory mechanism should enable the system to respond quickly to changes in its environment.

(6) Insensitivity to perturbations in the structure of the system itself. Small changes in the system may be the result of mutations in structural or regulatory genes, errors in transcription or translation of the genetic information, or physical influences such as temperature shifts. These changes tend to exert a deleterious effect on the cell and, as Sonneborn suggested[33], cells "buffered" against such harmful effects are likely to have a selective advantage.

Repressor-controlled systems

Models of classical and autogenous regulation are represented schematically in Fig. 1. In the classical Jacob–Monod model the structural gene for the regulatory protein is located in a separate transcriptional unit and is itself unregulated, whereas in the model of autogenous regulation one of the protein products of the operon is the regulator, which therefore directly controls expression of its own structural gene. For purposes of comparison these models are assumed to be equivalent except for their differences in regulatory interaction.

These two models can be compared for a wide variety of conditions, and because there are several criteria to consider, a meaningful tabulation of all the results presents a problem. Figure 2, however, is a rather simple diagram that conveniently summarises many of these results. In this plot the vertical axis represents the strength of the contribution of end product to the regulation while the horizontal axis represents the strength of the autogenous contribution. Each point in this two-dimensional space represents a different system with distinctive properties. Nevertheless, this space can be divided into subclasses of systems that have similar properties.

In repressible systems that utilise a repressor mechanism, the end product has a negative effect on transcription, and the autogenous contribution is also negative. Since both contributions are negative, only the lower left hand quadrant of Fig. 2 needs to be considered. The vertical axis in this quadrant is the locus of points representing systems with constitutively synthesised repressor, since the autogenous contribution is zero Line *b* represents another important classification. The position of this line is determined by analysing the local stability of these models. All systems represented by points above line *b* are stable (if perturbed momentarily, they will return to their predisturbance condition), whereas all systems represented

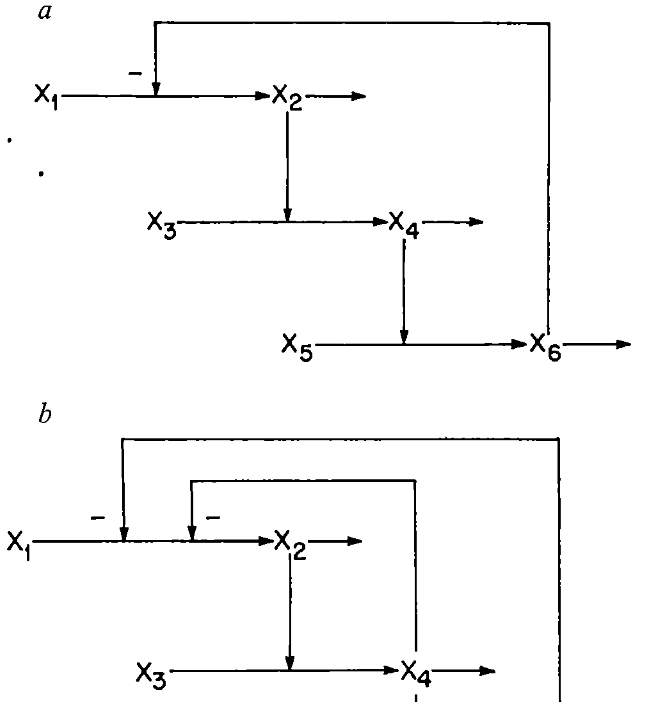

Fig. 1 Schematic models of repressible biosynthetic systems. Model with constitutive synthesis of the regulatory protein, which is not explicitly indicated. *b*, Model with autogenously regulated synthesis of the regulatory protein. X_1, Weighted sum of the nucleotide pools; X_2, specific messenger RNA; X_3, weighted sum of the amino acid pools; X_4, enzymes of the biosynthetic pathway (plus regulator in the case of autogenous regulation); X_5, substrate, and X_6, product/modifier. The horizontal arrows indicate chemical transformations at the mRNA, enzyme and metabolite levels. The vertical arrows represent modifier or catalytic influences (the sense of the influence is positive unless otherwise indicated). Except for the differences in regulation these models are assumed to be identical.

below this line are unstable (they will not return to their predisturbance condition).

Lines, such as *c*, *d* and *e*, that radiate from a single point on this plot are lines of equivalence with respect to the first three criteria for functional effectiveness. All systems whose regulatory parameters have values that determine points on a given line of equivalence have: (1) the same steady-state response to a change in demand for the end product, (2) the same steady-state response to a change in substrate availability and (3) the same decrease in enzyme synthesis for a given increase in the concentration of extracellular end product. The slope of the line determines the magnitude of the above three responses. Systems represented on a steeper line of equivalence, function more effectively according to the first and third criteria but less effectively according to the second

Systems for which the second criterion is of primary importance are represented by lines of equivalence with low slope, for example, line *c*. As previously noted, all systems on such a line have identical responses according to the first three criteria. Comparisons on the basis of stability, the fourth criterion, can be made by noting the relative distances of points on line *c* from the boundary of instability, line *b*, in Fig. 2. Since lines *c* and *b* diverge to the left, systems with greater strengths of autogenous regulation are represented further from the boundary of instability than is the equivalent system with constitutive synthesis of repressor. Consequently, this latter system is the least stable of all the systems represented on the line of equivalence *c*.

Systems for which the first three criteria are of equal importance are represented by lines of equivalence with intermediate slope, such as line *d* in Fig. 2 All the systems represented on this line have identical responses according to the first three criteria. They also have the same degree of stability since line *d* is parallel to the boundary of instability, line *b*.

Similarly, systems for which the first and third criteria are of primary importance are represented by lines of equivalence with steep slope, for example, line *e* in Fig 2 These systems are equivalent as judged by the first three criteria, but the systems with autogenously regulated synthesis of repressor must be considered inferior to the equivalent one with constitutive synthesis of repressor according to the fourth criterion because autogenous regulation now is clearly a destabilising influence

The two types of repressor-controlled systems are examined in Fig. 3 on the basis of temporal responsiveness, the fifth criterion for functional effectiveness. The three panels in this figure correspond to the three different classes of equivalence represented by lines *c*, *d* and *e* in Fig. 2. In the top panel (*c*) the response labelled 0.0 corresponds to the system with constitutive synthesis of repressor. The system is in a steady state with concentrations normalised to unity before time zero. At $t=0$ the concentration of extracellular end product is increased and then maintained at an elevated level throughout the experiment. The concentration of intracellular end product is plotted as a function of time. Similar responses are plotted for a series

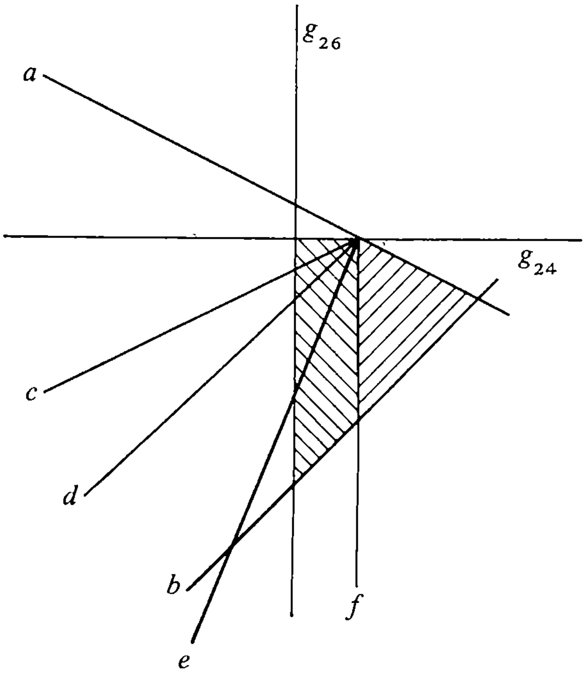

Fig. 2 Graphical comparison of systems with alternative types of regulation. The vertical axis represents the strength of the product's contribution to the regulation of transcription and the horizontal axis represents the strength of the autogenous contribution to this regulation *a* and *b*, Boundaries of instability. *c*–*f*, Lines of equivalence with respect to the first three criteria for functional effectiveness The vertical axis is the locus of points representing systems with constitutive synthesis of either activator or repressor. The shaded areas to the right of this axis are the locations of points representing stable systems with autogenously regulated synthesis of activator. Similarly, the area above line *b* in the lower left hand quadrant represents stable systems with autogenously regulated synthesis of repressor. (See text for further discussion)

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of systems with different strengths of the autogenous contribution to the regulation. Clearly, autogenous regulation decreases the time required for the system to settle into the new steady state. The same is true in Fig. 3*d*. In Fig 3*e*, however, autogenous regulation actually increases the response time.

A comparison on the basis of the sixth and final criterion for functional effectiveness shows that the sensitivities to external perturbations of the systems with autogenously regulated synthesis of repressor are less than or equal to those of the system with constitutive synthesis of repressor.

On the basis of the comparisons discussed above one can draw the following conclusions. If the second criterion for functional effectiveness is of primary importance, then systems with autogenously regulated synthesis of repressor will have a selective advantage. The behaviour of these systems is superior to that of systems with constitutive synthesis of repressor on the basis of the second, fourth, fifth and sixth criteria, but inferior with regard to the first and third. But if the first and third criteria for functional effectiveness are of primary importance, as seems more reasonable for biosynthetic systems, then systems with constitutive synthesis of repressor will have the selective advantage. The behaviour of these systems is superior to that of systems with autogenously regulated synthesis of repressor on the basis of criteria one, three, four and five, but inferior with regard to two and six. These conclusions will be discussed in relation to available experimental evidence in a later section.

Activator-controlled systems

The models represented in Fig. 1 also can be used for the comparisons in this section. Systems with constitutive synthesis of an activator protein can be represented directly by the model in Fig. 1*a*, whereas systems with autogenously regulated synthesis of activator can be represented by the model in Fig. 1*b* provided the negative sign previously associated with the repressor is changed to a positive one, implying activation. Since the autogenous contribution to the regulation now is positive, only the lower right hand quadrant of Fig 2 need to be examined. The systems with constitutive synthesis of activator are represented along the vertical axis. As in the previous section, line *b* represents the lower boundary of stability. In addition, there is an upper boundary of stability given by line *a*. As in the previous section, lines, such as *c*, *d*, *e* and *f*, that pass through a common point on this plot are lines of equivalence with respect to the first three criteria for functional effectiveness.

Systems for which the second criterion is of primary importance again are represented by points on line *c*. These systems are equivalent with respect to the first three criteria, but since line *c* diverges to the left from the boundary of instability, line *b*, the system with constitutive synthesis of activator is more stable than the equivalent systems with autogenously regulated synthesis of activator Systems for which the first three criteria are of equal importance are represented by points on a line of equivalence with intermediate slope, such as line *d*. All the systems represented on this line have identical responses according to the first three criteria, and according to the fourth criterion as well, since lines *b* and *d* are parallel. Similarly, systems for which the first and third criteria are of primary importance are represented by a steeper line of equivalence, such as *e* in Fig. 2. These systems are also equivalent by the first three criteria, but those with autogenously regulated synthesis of activator must be considered superior to the equivalent one with constitutive synthesis of activator according to the fourth criterion because autogenous regulation is a stabilising influence. Thus, the effect of autogenous regulation on the stability of activator-controlled systems is just the opposite of that found to be true for repressor-controlled systems.

The results in Fig. 4 show that autogenous regulation also has opposite effects on the response times of repressor-controlled and activator-controlled systems. The three panels in this figure correspond to the three different classes of equivalence represented by lines *c*, *d* and *e* in Fig. 2 The response time is increased by autogenous regulation in Fig. 4*c* and *d* In Fig. 4*e*, however, the response time is decreased by autogenous regulation.

A comparison on the basis of system sensitivity, the sixth criterion, shows that the sensitivities to external perturbations of the system with constitutive synthesis of activator always are less than or equal to those of the equivalent systems with autogenously regulated synthesis of activator.

The following conclusions can be drawn from the comparisons in this section. If the second criterion for functional effectiveness is of primary importance, then systems with constitutive synthesis of activator will have a selective advantage. These systems are superior to systems with autogenously regulated synthesis of activator by the second, fourth, fifth and sixth criteria, but inferior according to the first and third. If, however, the first and third criteria for functional effectiveness are of primary importance, as seems more reasonable for biosynthetic systems, then systems with autogenously regulated synthesis of activator will have the selective advantage. These systems are

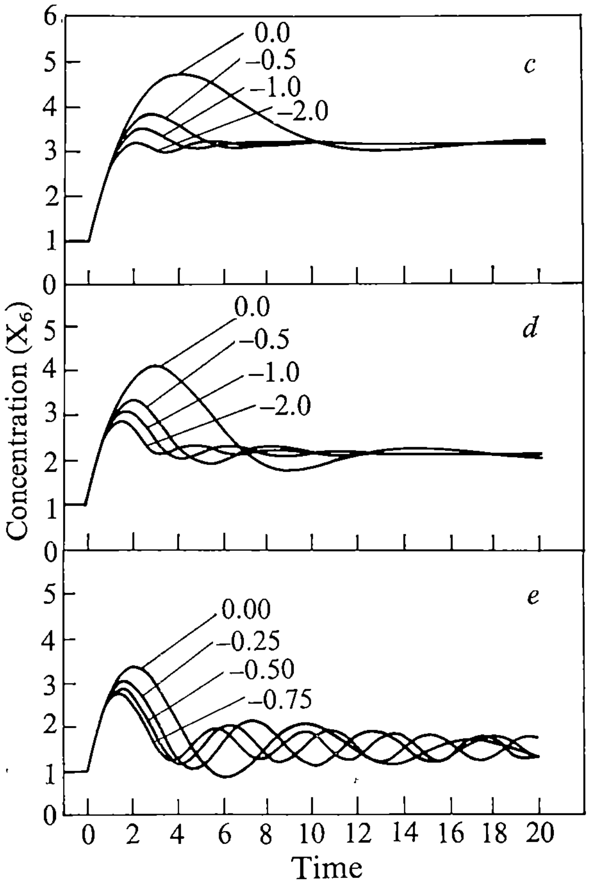

Fig. 3 Temporal responsiveness of systems with autogenously regulated synthesis of repressor. *c*, *d* and *e* correspond to systems in the three different equivalence classes represented by lines *c*, *d* and *e* in Fig 2. The numbers associated with the individual curves are values of g_{24}, representing the strength of the autogenous contribution to regulation. The corresponding values for the parameter g_{26} are chosen so that the points representing the different systems within a class all lie on the same line of equivalence. Curves with $g_{24} = 0$ represent systems with constitutive synthesis of repressor. (See text for further discussion.)

superior to systems with constitutive synthesis of activator on the basis of criteria one, three, four and five but inferior with regard to two and six.

(Re-examination of the lower right-hand quadrant in Fig. 2 shows that only a portion of the allowable systems have been considered in the preceding comparisons. The remaining systems with autogenously regulated synthesis of activator exhibit two classes of behaviour for which there is no counterpart among the other types of regulatory systems considered here Line *f* represents the class of systems for which there is zero depletion of end product in steady state after an increase in demand for end product. The class of systems represented by points within the shaded triangular region to the right in Fig. 2 have the unique property of responding to an increased demand for end product by increasing the level of that end product—a response to demand that is the inverse of the usual one. Whether such behaviour has biological significance is unclear and it will not be discussed further here A more detailed analysis is given in ref. 31.)

Predictions and observations

As previous sections of this article show, the effectiveness of activator-controlled and repressor-controlled operons is changed in opposite ways if the regulator protein is, in addition, autogenously regulated. In systems for which the first and third criteria for functional effectiveness are most important, as is likely for repressible biosynthetic systems, autogenous regulation of the regulator protein improves the performance of activator-controlled systems and degrades that of repressor-controlled systems. Thus, biosynthetic systems governed by a repressor are not expected to use autogenous regulation, while those governed by an activator can be predicted to involve such regulation. These predictions were deduced from differences inherent in the classical and autogenous modes of regulation, other things being equal. Present information concerning actual, specific operons is insufficient to yield such generalisations through induction from individual cases. Nevertheless, the experimental evidence is consistent with the above predictions in the few cases where data that can be analysed are available.

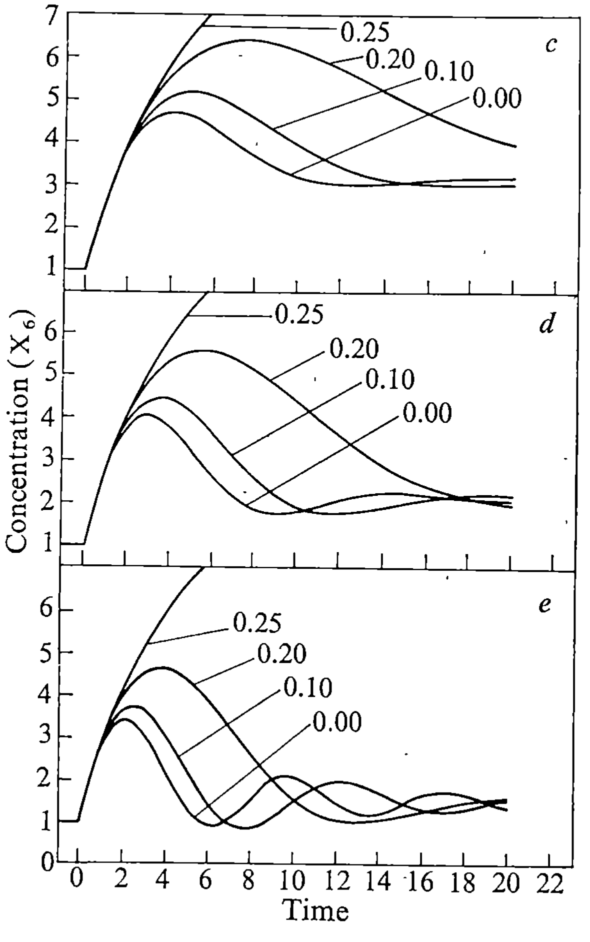

Fig. 4 Temporal responsiveness of systems with autogenously regulated synthesis of activator. *c*, *d* and *e* correspond to systems in the three different equivalence classes represented by lines *c*, *d* and *e* in Fig. 2 The numbers associated with the individual curves are values of g_{24}, representing the strength of the autogenous contribution to regulation. The corresponding values for the parameter g_{26} are chosen so that the points representing the different systems within a class all lie on the same line of equivalence. Curves with $g_{24} = 0$ represent systems with constitutive synthesis of activator. (See text for further discussion.)

In the case of the tryptophan operon in *E. coli*, the best documented example of a biosynthetic system involving control by a repressor, there is no evidence for autogenous regulation of repressor. The structural gene for the tryptophan repressor is located outside the known transcriptional units under its control[34], so the simplest form of autogenous regulation is excluded. Nevertheless, a more complex, regulon model, in which the repressor modulates expression of its own structural gene in parallel with that of the tryptophan operon, cannot be excluded rigorously since repressor levels have not been compared in strains with the tryptophan operon repressed and derepressed.

In the case of the arginine system in *E. coli*, which also seems to involve control by a repressor, there is no evidence for autogenous regulation of repressor. Although the arginine system itself constitutes a regulon with several unlinked transcriptional units regulated in parallel[35], the structural gene for the repressor is not linked to any of these[34] and there is no evidence that its expression is subject to modulation by the level of arginine within the cell.

On the other hand, the clearest example of a biosynthetic system involving control by an activator is the isoleucine–valine system in *Saccharomyces cerevisiae* and in this case the activator is autogenously regulated[36,37]. With an extensive collection of mutants in the structural gene for the first enzyme of the isoleucine pathway (threonine deaminase), Bollon[37] has constructed a fine-structure map and demonstrated that this protein is multifunctional; mutations affecting its activator function overlap with mutations affecting its catalytic function.

For all three operons discussed above the evidence is consistent with the predictions based on the analysis of autogenous regulation. For other biosynthetic systems the experimental evidence is more ambiguous. For example, in *E. coli* there is strong experimental evidence that the first enzyme of the isoleucine pathway is involved in the autogenous regulation of the isoleucine–valine system, but whether it functions as repressor or activator is unclear. The prediction based on the analysis in the preceding sections is that some product of the operon is an activator, in agreement with the data of Levinthal *et al.*[28]. Similarly, in *S. typhimurium* there is evidence consistent with the idea that some product of the histidine operon is involved in autogenous regulation of the system, but whether this product is an activator or a repressor is controversial. Again, the prediction is that this product is an activator, in agreement with the data of Artz and Broach[22]. (The conflicting evidence for the nature of the regulators of these operons, which was referred to in the introduction, might be reconciled more easily if they were in fact biregulators rather than activators It is easier to conceive of a biregulator changing physical state, as a result of mutation or environmental conditions, and exhibiting the properties of either repressor or activator than to imagine an activator doing this. Nothing in the analysis and predictions given above would change substantially if activators were considered biregulators analogous to the arabinose *C*-gene product, which is primarily an activator in function but also has repressor attributes. The arguments are given in ref. 31.)

The tryptophan and arginine biosynthetic systems have been described in terms of a classical, Jacob–Monod model with constitutive synthesis of regulator. On the other hand, the histidine and isoleucine–valine biosynthetic systems have been described in terms of an autogenously regulated model with an enzyme/regulator directly involved in modulating expression of its own structural gene. These two models, however, need not be mutually exclusive. A combination of the two modes of regulation might exist, possibly with one or the other mode playing a dominant role in some systems. The results in the preceding sections suggest that the most promising of such combinations would be constitutive synthesis of a repressor and autogenously regulated synthesis of an activator. Is there any experimental evidence for such a combination?

There is evidence for multiple control mechanisms in most of the biosynthetic systems that have been well studied. Mutants with a chain-termination codon early in the structural gene for the first enzyme or with this gene deleted have been described in the histidine[19] and isoleucine–valine[38] biosynthetic systems of enteric bacteria. In each case, the system continues to be regulated in an apparently normal manner, at least by the usual all-or-none tests for regulatory function. Thus, mutations that eliminate the autogenous mode of regulation permit another, possibly the classical mode, to become manifest.

A better example is provided by the tryptophan operon of *E. coli* In this system, where there is clear evidence for the classical mode of regulation involving a constitutively synthesised repressor, there also is evidence suggesting that the first enzyme of the tryptophan pathway (anthranilate synthetase, EC 4 1 3.27) is involved in regulating expression of the operon [39]. Somerville and Stetson[40,41] reported additional evidence for involvement of the first enzyme in regulation of the tryptophan operon and suggested that this enzyme is normally an activator or positive element in the control, which agrees with the above prediction. When the structural gene for the first enzyme is deleted, the tryptophan operon continues to be regulated in an apparently normal manner, again by the usual all-or-none tests for regulatory function[42]

In addition to the two modes of regulation discussed above there are indications of novel control mechanisms[43–45], at least some of which involve sites on the DNA between the promoter–operator region and the first structural gene of the operon[44,45].

In systems for which the first and third criteria for functional effectiveness are relatively unimportant, the results are just the reverse of those discussed above Autogenous regulation of the regulator protein improves the performance of repressor-controlled systems and degrades that of activator-controlled systems Although it seems unlikely that any repressible biosynthetic system would fall into this particular class, there are systems of other types that might be considered non-biosynthetic, that do fit this category. For example, Sompayrac and Maaløe[46] have proposed a model for regulating the concentration of the protein(s) believed to be involved in the initiation of DNA replication The regulator protein in this model is autogenously regulated and functionally autonomous, that is, it does not require the participation of an effector molecule in its regulatory function. Although not a repressible biosynthetic system in the conventional sense of the term, formally this model is a special case of that represented in Fig. 1*b* with $g_{26} = 0$. Accordingly, such systems would be represented along the horizontal axis in Fig. 2 Sompayrac and Maaløe[46] pointed out several advantages for their model involving a repressor whose synthesis is autogenously regulated. The results in the preceding sections suggest that the behaviour of such systems is optimal with respect to stability, temporal responsiveness and insensitivity to perturbations in the structure of the system itself. Dennis and Nomura[47] have indicated that in *E. coli* a similar mechanism of autogenous regulation might be involved in control of ribosomal protein genes.

Perhaps it should be re-emphasised here that the predictions made throughout this article are based on a particular weighting of the different criteria for functional effectiveness. If this choice were incorrect, then the predictions could be wrong, but clearly for reasons that have nothing to do with the general validity of the approach used.

In conclusion, the expression of biosynthetic operons in enteric bacteria is undoubtedly controlled by several different mechanisms acting in concert This has made it difficult to study regulation *in vivo* where all the elements may interact or contribute in various degrees to produce the end result. The identity of the elements participating in the regulation and the nature of their roles at the molecular level can only be established rigorously *in vitro*. An understanding of the intact regulatory system, however, will require knowledge of the functional implications at the physiological level for each molecular mechanism and, eventually, integration of the individual mechanisms into an accurate model of the intact system With progress being made in each of these areas, the understanding of repressible biosynthetic operons should soon be as thorough as it is of the simpler inducible catabolic operons.

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letters to nature

Soft X-ray search of centre of Cygnus Loop

THE Mullard Space Science Laboratory equipment on the Copernicus satellite has been used to search, with negative results, for evidence of a compact object in the centre of the Cygnus Loop supernova remnant.

Rocket measurements[1] indicate that a central object exists, and contributed, at the time of observation, about 4% of the X-ray flux from the entire loop. This object may be variable[1,2] in intensity, and may even be pulsing[1]. Reanalysis of earlier rocket data[3] gives statistically more significant evidence for pulsation, but at a different frequency, which would be consistent with a compact object which is not a pulsar. The same data require, however, that the pulsing object be roughly equal in brightness to the integrated brightness of the rest of the nebula at the relevant energy, 0.28 keV, at the time of observation, while other rocket data[4,5] show that this is certainly not the case at particular other times. The availability of a Channel Electron Multiplier (CEM) detector, sensitive at the relevant energy (0.28 keV), on board the Copernicus satellite, has provided the opportunity to observe the centre portion of the Cygnus Loop and to test whether a central object does, from time to time, radiate as strongly at 0.28 keV as rocket data[3] suggest.

The CEM has been described by Margon *et al.* (see ref. 6 and references therein). The field-of-view of the CEM is 15′ full width at half maximum. Since the exact position of the central object is not known, a set of nine observing sections, centred on the rocket[1] position, were chosen so that there was a slight degree of overlap. These nine positions are given in Table 1.

The observations were made on May 19, 1974. Three spacecraft orbits were required to observe the nine sections. Sections, 1, 2, 3 were covered from 1349 to 1422 UT; 6, 5, 4 from 1530 to 1600 UT; and 7, 8, 9 from 1709 to 1739UT. The observations could only be made while the satellite was in the shadow of the Earth since the CEM is also sensitive to Lyman alpha (Lα) radiation. During the periods when the satellite was in sunlight, the CEM was turned off. (Margon *et al.*[6] note that in spite of the Lα background, the telescope is known to respond normally to low energy X rays; in particular Sco X-1 has been observed.) Each data acquisition interval lasted 62.5 s. From six to ten observations were made in each section.

Figure 1*a* presents plots of the raw data as received from the satellite. Three points are immediately apparent: (1) each plot displays a characteristic parabolic shape, due to CEM sensitivity to geocoronal Lα radiation; (2) a systematic decrease in this background occurs on successive orbits, clearly due to instrumental effects; and (3) no sharp break occurs between any of the three sections on each of the three orbits, as would be expected if a strong source of 0.28 keV X rays were present. If the central source were present, at the greatest intensity observed[3], we would expect that one of the sections would display a 'discontinuity' of about 735 counts over the Lα background, given the known sensitivity[6] to X rays of the CEM. A lower flux would result in a smaller discontinuity. The data show that if such a source was present in the field of view, it was not of high intensity at the time of observation.

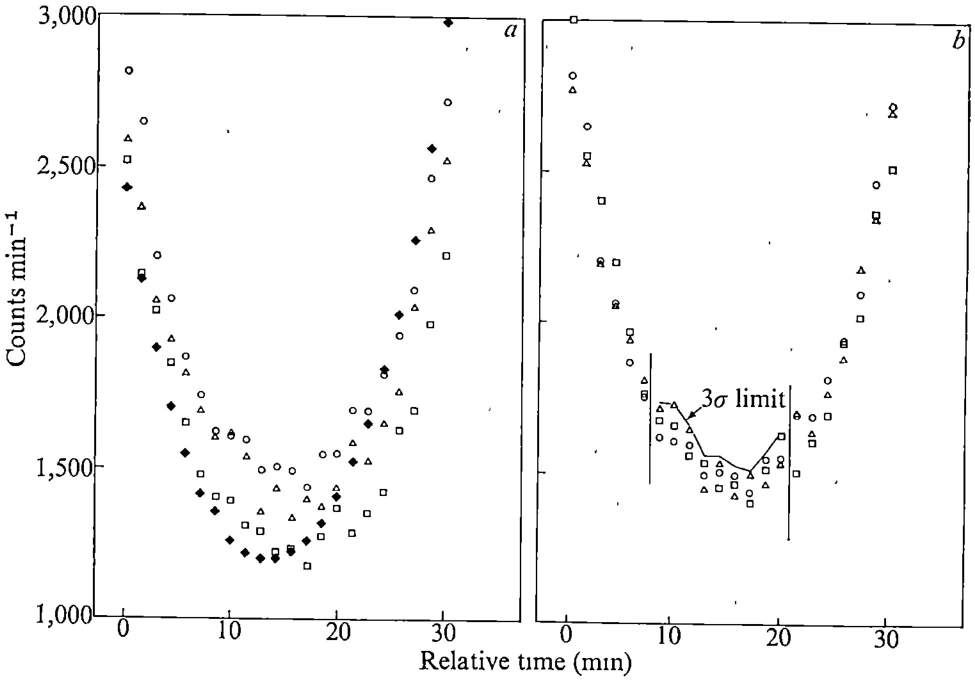

Fig. 1 *a*, Signal received from directions near the centre of the Cygnus Loop nebula on three orbits of the Copernicus satellite. The data are shown in raw form, and give no evidence for any soft X-ray emission. Also shown is the relative expected background of Lα radiation, calculated by Dr R. Meier of the US Naval Research Laboratory. *b*, The data of *a* are shown, processed to remove instrumental effects. This permits the determination of a 3σ upper limit on any possible X-ray source, which is about 80 times smaller than the highest reported observed intensity. ○, First orbit; △, second orbit; □, third orbit; ◆, theoretical Lα.

The change in sensitivity of the CEM would be unimportant if an obvious discontinuity in the data was apparent. One could say in those circumstances that a source was present, and one could determine in which section it appeared. In obtaining an upper limit for the source, however, the change in sensitivity becomes critical. Since each orbit displays the same characteristic pattern, we feel safe in believing that each curve represents only Lα background. We have therefore transformed orbit two and orbit three to orbit one by using a simple *ad hoc* function that corrects for the CEM drift. Almost any transformation will be acceptable if it results in a variance at each point comparable to some typical variance of the data points on one orbit about some smooth function representing the Lα background.

Figure 1*b* represents the result of using a correction consisting simply of multiplication of the data by an orbit-dependent constant. The constant for each orbit was chosen so that

$$\sigma^2 = \sum_{i=1}^{N} \sigma_j{}^2 = \sum_{j=1}^{N} \sum_{i=1}^{3} \left\{ \frac{(x_{ij} - \bar{x}_j)^2}{2} \right\} \qquad (1)$$

was a minimum where $\bar{x}_j$ is the average of the three transformed points, x_{ij} is the transformed value of point j in orbit i, and N observations are made during one orbit.

Table 1 Observed positions in the Cygnus Loop

Section	Position (1950) RA (h min)	Dec. (° ′)
1	20 50.5	+30 53
2	20 49.7	30 53
3	20 48.9	30 53
4	20 50.5	30 43
5	20 49.7	30 43
6	20 48.9	30 43
7	20 50.5	30 33
8	20 49.7	30 33
9	20 48.9	30 33

The minimum σ^2 was obtained by multiplying the observations of orbit 2 by 1.07 and of orbit 3 by 1.19, and each $\sigma_j{}^2$ was indeed comparable to the scatter among individual points observed on a single orbit, indicating that no more complicated correction procedure is justified.

A three sigma upper limit on the intensity of any soft X-ray source may be obtained by adding a source of ε photons per 62.5 s to the background Lα radiation in one of the sections in Fig. 1*b*. The value of ε is increased until the quantity

$$\chi^2 = \sum_{j=1}^{n} \frac{((\bar{x}_j + \varepsilon) - \bar{x}_j)^2}{\sigma^2{}_j} \qquad (2)$$

(where n is the number of observations in a section), becomes so large that one can say with 99.74% certainty that the data in that section include something more than Lα radiation. The line in Fig. 1*b* represents this three sigma limit.

Knowing the relevant parameters of the CEM, a three sigma upper limit on the intensity of the X-ray source of 5.7 photons (cm^2 s keV)$^{-1}$ is obtained. This is a factor 80 below the highest reported rocket intensity[3].

This negative result could indicate that the X-ray source was simply not in its high-intensity mode at the time of observation, or could arise because the source is at some other location in the Loop. The possibility also remains, of course, that the evidence for a central object[1], intensity variations[1,2], and pulsations with very high intensity[3], is misleading and no such object exists If it does exist, it is clear that it is strongly time-dependent, and it would be valuable to monitor the assumed source position frequently. Unfortunately noise bursts on the CEM channel have precluded additional observations.

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Proper motions of six pulsars

SEVERAL authors have suggested that pulsars may be high velocity objects. Prentice[1] found, from a study of the wide distribution of the distances of pulsars to the galactic plane, that their initial velocities may have been as high as 100 km s^{-1}, and Michel[2] obtained a similar figure for runaway objects formed by the collapse of core fragments in supernova events. Tademaru and Harrison[3] proposed a mechanism for the acceleration of pulsars, by asymmetric radiation, to velocities of 1,000 km s^{-1} Only rather meagre direct experimental evidence for high velocities exists, however. This evidence is derived from three different types of observation. First, Lang and Rickett[4], Galt and Lyne[5], and Slee *et al*[6] have deduced velocities of between ~ 40 km s^{-1} and ~ 600 km s^{-1} from measurements of the velocity of the interstellar scintillation pattern, found by comparing the scintillation observed at two widely separated stations. These conclusions rely, however, on the pattern being stable in time. In some cases this is clearly not so, and in particular Uscinsci[7] has shown how shear within the scintillating medium can produce an apparent velocity which bears little relation to that of the pulsar. These measurements must therefore be treated with caution Second, pulse-timing studies by Manchester, Taylor and Van[8] gave an apparent motion of 0 58±0.22″ for PSR1133+16, corresponding to a velocity of 380 km s^{-1}. Finally, the transverse velocity of the optical object identified with the Crab Nebula pulsar, PSR0531+21, is 100 km s^{-1} as deduced from the proper motion and estimated distance[9].

Here we report measurements on six pulsars which seem to have motion velocities at least five times greater than the formal standard errors Measurements were made on a radio link interferometer of 127-km baseline, using the Mk 1A 76-m radio telescope at Jodrell Bank and a 25-m

Table 1 The proper motions observed for six pulsars

Pulsar	$\dot{\alpha}\cos\delta$ ($'' \text{yr}^{-1}$)*	$\dot{\delta}$ ($'' \text{yr}^{-1}$)*	$\dot{r}$ ($'' \text{yr}^{-1}$)*	Position angle (degrees)*	Distance (pc)	Velocity (km s^{-1})	Angle φ between $\dot{r}$ and galactic plane (°)
0329+54	0.011 ± 0.006	-0.008 ± 0.004	0.014 ± 0.004	126 ± 16	2,500	166	$+1.5\pm16$
0823+26	0.050 ± 0.014	-0.125 ± 0.010	0.135 ± 0.010	158 ± 6	649	416	$+5\pm6$
0834+06	-0.017 ± 0.018	0.049 ± 0.014	0.052 ± 0.014	-19 ± 20	430	106	$+8\pm20$
1133+16	-0.061 ± 0.036	0.360 ± 0.036	0.365 ± 0.036	-10 ± 8	162	281	$+26\pm8$
1237+25	-0.098 ± 0.018	0.027 ± 0.014	0.102 ± 0.018	-75 ± 8	308	149	-33 ± 8
2016+28	0.003 ± 0.012	0.020 ± 0.008	0.020 ± 0.008	9 ± 26	473	45	-47 ± 26

* $\pm2\sigma$

antenna at Defford, Worcestershire, used by arrangement with the Royal Radar Establishment, Malvern. The observing frequency was 408 MHz, with an operating bandwidth of 4 MHz. A phase reference technique similar to that described by Peckham[10] was used, in which the pulsar and a small diameter background radio source are observed simultaneously within the same telescope beam. The relative phases of the two objects are compared at different epochs and, assuming that the background source is extragalactic, any change in the relative phase is then attributed to a proper motion of the pulsar.

Observations were made in November 1973, July 1974, November 1974 and April 1975. Seven pulsars were observed in 1973, and these and a further seven in the subsequent sessions. At each epoch, each pulsar was observed for as wide a range of hour angles as possible when its elevation was greater than 10°. If time permitted, each observation was repeated.

Table 1 shows the proper motions tabulated in both celestial coordinates and polar coordinates. The velocities given in the final column were calculated using the distances presented in the penultimate column. These distances were obtained from the dispersion measures, assuming an average electron density of 0.03 cm^{-3} (refs 11 and 12), except in the case of PSR0329+54, for which absorption measurements place the pulsar on the near side of the Perseus arm of the galaxy (R. S. Booth and A. G. Lyne, unpublished). This use of the dispersion measure is somewhat uncertain, but we believe that it is justified since Prentice and ter Haar[13] found no regions of high ionisation along the line of sight to any of these pulsars. We have not used their distance estimates directly, because of the rather high mean electron density (0.05 cm^{-3}) used in their model. The discrepancies are not large, however.

The uncertainties quoted in Table 1 are all formal 2σ errors, and these are a function of the intensity of the source and the pulsar, and also of the observing time and hour angle coverage. These errors should be treated with caution, since ionospheric and interstellar scintillation effects can cause fluctuations in the relative-phase curves on time scales comparable with the length of an individual observation. Although the effects of these scintillations will average out as a larger number of observations are made, with the data obtained so far we do not believe the uncertainties can be much less than 0.01″ yr^{-1}. In the case of PSR0329+54, however, the measurements are expected to have less uncertainty, because it is a relatively intense pulsar with a strong background source, it was observed at high elevations, and several repeat observations were made.

The results for the two pulsars PSR0329+54 and PSR1133+16 show some agreement with previous measurements. In the case of PSR0329+54, the measured position angle of 126±16° agrees well with the value of 115±15° found by Galt and Lyne[5] from scintillation observations. This supports their second interpretation: that it is the pulsar, rather than the diffraction pattern, which is moving with high velocity They deduce a velocity which is more than a factor of two greater than the present measurement, but this could be explained if the diffracting screen were situated slightly nearer the pulsar, or if the distance has been underestimated. Our result for PSR1133+16 agrees, within the errors in both magnitude and direction, with that of Manchester, Taylor and Van[8].

The data in Table 1, together with that for the Crab Pulsar, PSR0531+21, show typical transverse velocity components in the range 100–200 km s^{-1}. Thus we may deduce that the resultant velocities are high, typically 150–300 km s^{-1}, although none is as high as the 1,000 km s^{-1} suggested by several authors[1–3]. It must be remembered, however, that we are, as yet, dealing with very small numbers of pulsars, and also that there are still some uncertainties in these velocities because of the uncertainties in the pulsar distances.

It is widely believed that pulsars originate in population I type objects which lie within about 100 pc of the galactic plane, and that their high velocities then carry them to heights of several hundred parsecs during their lifetimes. This picture is weakly supported by the data presented in column 8 of Table 1 which shows the angle ($-90° < \varphi < 90°$) between the velocity vector $\dot{\mathbf{r}}$ and the galactic plane. A positive sign indicates that the apparent motion is away from the galactic plane. It can be seen that three of the pulsars (PSR0823+26, PSR0834+06 and PSR1133+16) are probably moving away from the plane; the direction of motion of PSR0329+54 is not established accurately enough to ascertain whether it is receding from or approaching the plane; and PSR2016+28 and PSR1237+25 seem to be approaching the plane. PSR0329+54 and PSR2016+28 lie within the population I distribution, however, and PSR1237+25 is situtated at such a high galactic latitude (86.5°) that motion towards or away from the plane is likely to be dominated by the unknown radial component of velocity. The unknown radial components of velocity of the other pulsars also affect their apparent motions with respect to the galactic plane so that the evidence for recession from the plane is not very strong.

Manchester, Taylor and Van[8] have estimated a 'kinetic age' for PSR1133+16 by projecting back the apparent motion on the assumption that it started from the galactic plane and that it now has no radial component of velocity. The pulsar velocity should be substantially unaffected by the gravitational attraction of the galactic plane during the pulsar lifetime since the oscillation period of a star across the plane is in excess of 10^8 yr in the region of the Sun. Table 2 shows the 'kinetic ages' for the three pulsars which clearly seem to recede from the galactic plane together with the 'rotational age' derived from observations of changes in

Table 2 Kinetic and rotational ages of three pulsars

Pulsar	Kinetic age ($\text{yr}\times10^{-6}$)	Rotational age ($\text{yr}\times10^{-6}$)
0823+26	11	5
0834+06	14	3
1133+16	3.4	5

pulsar periods ($P/2\dot{P}$). The 'kinetic ages' should be regarded as probable upper limits to the true ages of the pulsars since the ages would be significantly reduced if the pulsars had radial components of velocity as small as $+60 \text{ km s}^{-1}$. Because of this, these 'kinetic ages' are not sufficiently accurate to test a recent suggestion by Lyne, Ritchings and Smith[14] that the 'rotational age' may be a substantial overestimate of the true age, particularly in the case of old pulsars.

It is worth noting that the proper motions which have been determined, so far, for pulsars—the six reported here and also the Crab Nebula pulsar, PSR0531+21 (ref. 9)—tend to lie parallel to the galactic plane rather than perpendicular to the plane. The probability of this occurring by chance is quite small, although so few measurements are available that the significance is difficult to assess.

Continuation of this work over longer time scales will yield improvements in accuracy, and provide measurements of a larger number of proper motions. These will be particularly useful in discriminating between genuine and false pulsar–supernova associations and can provide independent measurements of pulsar ages.

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Energy spectrum of ultra high energy cosmic rays

AT energies above ~ 10^{17} eV it becomes increasingly difficult to maintain the observed near isotropy of ultra high energy cosmic rays if they are of Galactic origin, unless there is an extensive halo magnetic field, and the primaries are heavy nuclei[1]; instead, extragalactic origin is often assumed. The models proposed can be divided into three main groups: (1) 'universal', in which there is a class of galaxies in the Universe (of which our own is not a member) which are roughly uniformly distributed, and which generate the ultra high energy particles; (2) 'universal plus supercluster enhancement'[2], in which the existence of a supercluster is assumed—the enhanced density of galaxies in the SC allows a larger fraction, than is its share by volume, to have come from this region; and (3) 'Supercluster trapping'[3], where energetic phenomena in the SC generate the particles which are then trapped for periods of perhaps 10^9–10^{10} yr.

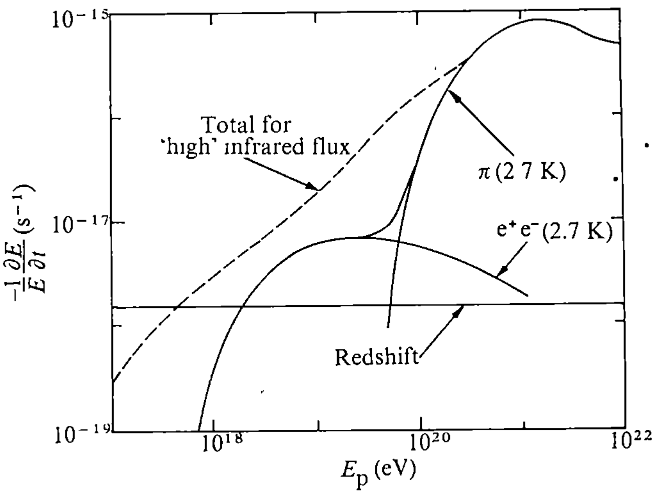

Fig. 1 Fractional rate of energy loss of protons. The solid lines represent the losses from π and e^+e^--production on the 2.7 K radiation field[2]. The dashed line is the aggregate rate of energy loss for the radiation field described in the text.

The problem with the universal model is that the universal black body radiation (2.7 K) should cause a rapid cutoff in primary intensity above about 6×10^{19} eV because of photomeson production[4,5], whereas the measured primary spectrum extends to 10^{20} eV (refs 6, 7 and papers in ref. 8), and perhaps to 3×10^{20} eV, without any indication of steepening. A similar situation exists in the 'supercluster trapping' model if the lifetime approaches the Hubble time.

We postulated the 'supercluster enhancement' model[2] in an effort to circumvent the difficulty by deriving quite a large fraction of the highest energy particle from nearby sources. There are difficulties in such a model, however, as we pointed out, because of the need to assume near-straight line trajectories; furthermore, there are questions about our membership of a supercluster (B. T. Jones, unpublished; J. N. Bahcall and P. C. Joss, unpublished).

The possibility of the extragalactic particles commencing their lives as heavy nuclei has been examined[9–12]. The conclusion is that heavy nuclei are not very helpful, but an important feature that has arisen from the examination of the propagation of heavy nuclei concerns the magnitude of the infrared component in extragalactic space. Puget and Stecker[12] derived so-called high and low infrared spectra. The 'high' infrared spectrum is quoted as $n(\varepsilon) = 1.1\times10^{-3}\ \varepsilon^{-2.6}$ (cm^{-3} eV^{-1}) valid for $2\times10^{-3}\text{ eV} < \varepsilon < 0.8\text{ eV}$ (giving a total energy density of $\sim 5\times10^{-2}$ eV cm^{-3}). There is no question of this being a definitive spectrum, in view of the paucity of observational data, but at least it is a possibility.

Our contribution now is to revert to the earlier situation[2] where extragalactic protons were considered. Figure 1 shows the fractional rate of energy loss for the case where only the 2.7 K radiation is considered and its modification when the 'high' infrared spectrum is included. It is now seen that the rapid rise in $(-1/E)\,(\partial E/\partial t)$ at $\sim 6\times10^{19}$ eV disappears, and instead there is a rather smooth variation over the whole range 5×10^{17}–10^{21} eV. The slope of the fractional rate of energy loss as a function of primary energy is almost unity, and this means that, for the universal model, if extragalactic sources have a production spectrum of the form E^{-2} then, above $\sim 5\times10^{17}$ eV, the spectrum at the earth will be close to that measured. (In this model the majority of particles below $\sim 5\times10^{17}$ eV are of galactic origin.) The assumption that extragalactic sources may have flatter spectra than that pertaining to our own Galaxy (for which the differential exponent is probably ~ 2.6: the measured exponent below ~ 10^{15} eV), is not unreasonable since these sources are more energetic.

For the supercluster enhancement model, there would again be agreement between observation and expectation; in fact there would be a flattening of the spectrum above about 10^{19} eV, where the SC contribution would start to dominate. It is pertinent to point out that there is indeed some evidence favouring such a flattening[13], a result that is also, incidentally, reasonable for a universal model in which sources having a variety of exponents contribute. Finally, the supercluster trapping model would give a very similar result to that for the universal model, if the particle lifetime is ~ 10^{10} yr. For a shorter lifetime the agreement would be reduced because the modulation caused by passage through the radiation fields would be smaller, although the infrared flux density within a supercluster will be higher than the universal average and some measure of compensation might be expected.

If the arguments advanced against the Galaxy belonging to the supercluster are correct and there is, in consequence, neither enhancement nor trapping, then the present suggestion seems to us to be one of the few ways of keeping a model with extragalactic protons. If later measurements show that the adopted infrared intensities are overestimates, then it is more likely that the ultra high energy particles are heavy nuclei of galactic origin and that there is an extensive halo.

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Solar flare effects in equatorial counter electrojet currents

THE solar flare effect on the horizontal component of the geomagnetic field (H) during the period of counter equatorial electrojet current is characterised by a negative crochet (decrease of the H field) at an equatorial electrojet station and a positive crochet (increase of H field) at a low latitude station outside the electrojet belt. During the period of partial counter electrojet, the crochet in H may be positive at all low latitude stations but the amplitude decreases at stations nearer to the equator. We explain this as the combined effect of the solar flare on the normal eastward currents over the Equator associated with Sq current system (around 107–110 km) as well as on the westward counter electrojet current (~100 km) both flowing simultaneously over the low latitude stations, while at stations outside the equatorial region the solar flare effect is due to only the eastward Sq currents. (The Sq currents are currents in the upper atmosphere arising from convective motion through the geomagnetic field[1,2].)

McNish[3] has shown that disturbances in the geomagnetic field during a solar flare (crochet effect) were similar in direction and magnitude to the normal Sq vectors, suggesting that the crochets arise because of the intensification of the Sq current from additional ionisation by the radiations from the solar eruptions. Subsequent analyses, however, showed systematic differences between the current patterns of the crochet and the Sq field[4]. These differences led to the idea that normal Sq currents are restricted mainly to the E-region, but during the period of solar flare the ionospheric currents extend down to the D-region of the ionosphere.

Nagata[5] showed that the magnitude of the crochet in the H field is abnormally large at Huancayo and at other low-latitude stations. Forbush and Casaverde[6] showed that the amplitude of crochets in H at Peruvian stations varied with latitude in a manner similar to the Sq(H) range. At times the electrojet currents over a low-latitude station during the daytime are found to be reversed, that is, the currents flow westward causing a depression in the H field. These events called counter-electrojet are closely associated with the various phenomena on the lower as well as the upper ionosphere[7]. Rastogi[8] postulated the simultaneous existence of Sq currents at altitudes of ~107 km over the equatorial zone always flowing eastward and other currents at ~100 km flowing either westward or eastward. The net change in the geomagnetic H field at ground level is produced by the effects of both the current systems. If the solar flare somehow increases the

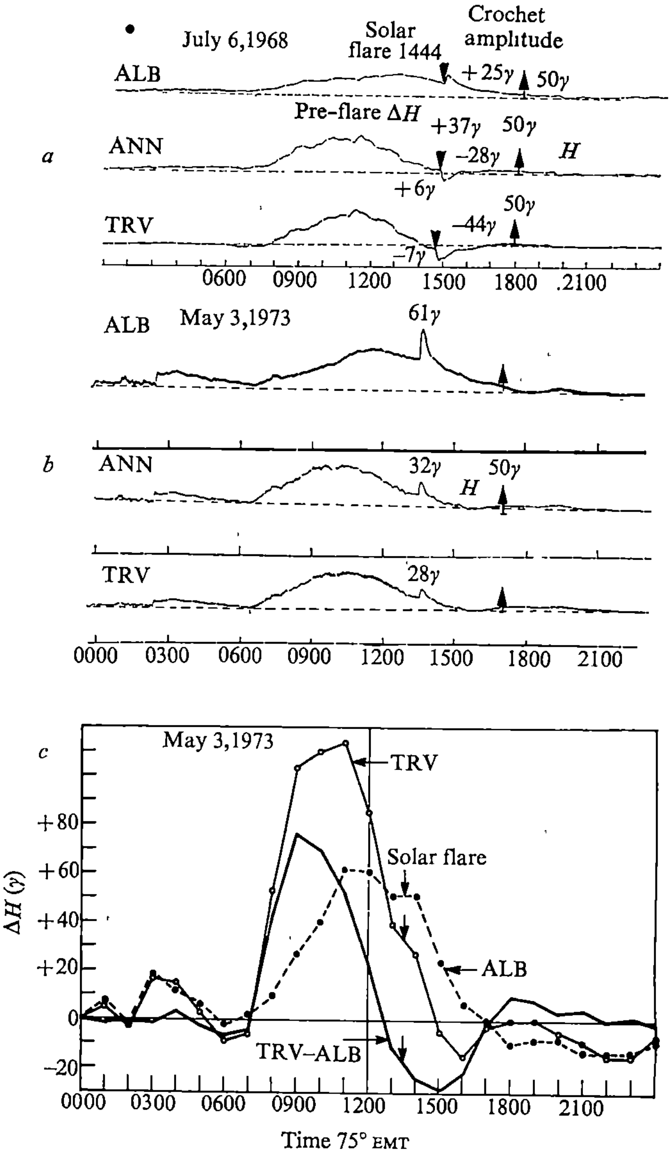

Fig. 1 *a*, Crochet effect in H at Indian stations during a complete counter-electrojet event on July 6, 1968 at Trivandrum (TRV), Annamalainagar (ANN), and Alibag (ALB). *b*, Crochet effect in H at Indian stations during a partial counter-electrojet event on May 3, 1973. *c*, Diurnal variations of the geomagnetic H field at TRV, ALB and TRV—ALB on May 3, 1973.

existing currents in the ionosphere, the effects of solar flares during periods of counter equatorial electrojets should be interesting. Here we examine magnetograms from Trivandrum (dip 0.6° S), Annamalainagar (dip 6°N) and Alibag (dip 25°N) for 1958–1973.

Figure 1*a* and *b* show magnetograms from these observatories during solar flares on July 6, 1968 at 1444 LT, when there was a clear counter electrojet, and on May 3, 1973 at 1330 LT, when there was a partial counter electrojet. Figure 1*c* shows the variations of the *H* field at Trivandrum, Alibag and the difference of the field between the two stations.

On July 6, 1968 the field at Trivandrum was below the night level when the flare occurred. The pre-flare ΔH was -7γ at Trivandrum, $+6\gamma$ at Annamalainagar and $+37\gamma$ at Alibag. The solar flare produced a change of -44γ at Trivandrum, -28γ at Annamalainagar and $+25\gamma$ at Alibag. Thus the crochet in *H* had opposite signs at stations within the electrojet belt and outside it, if the flare there was a counter electrojet.

For the solar flare on May 3, 1973, Srivastava[9] noted that the magnitude of crochet in *H* was larger at Hyderabad (dip 21°N) than at the equatorial stations. The magnitude of ΔH due to the solar flare was 28γ at Trivandrum, 32γ at Annamalainagar and 61γ at Alibag. Thus the crochet amplitude in *H* increased with increasing distance from the Equator, as opposed to the nature of the latitudinal variation Equatorial stations[10].

Figure 1*c* shows that after sunrise, *H* increased faster at Trivandrum than at Alibag until about noon. After 1230 LT, ΔH was smaller at Trivandrum than at Alibag. The ΔH(TRV−ALB) was negative from about 1230 to 1700. It has been shown that when ΔH at an equatorial station is positive, but the difference between this and ΔH at a station outside the electrojet region is negative, then the reversal of ionospheric drifts as well as the disappearance of plasma irregularities at the base of the ionospheric E region indicate a westward electric field, and so a counter-electrojet current is inferred[8]. Here, the *q* type of sporadic *E* at Kodaikanal had disappeared on the ionogram at 1245 LT precisely when ΔH (TRV−ALB) had reduced to zero and about half an hour before the occurrence of the flare. These facts clearly indicate the existence of the westward currents at the base of the *E*-region at the time of the occurrence of crochet on May 3, 1973.

At the stations outside the equatorial electrojet currents, Hyderabad and Alibag, where only the normal Sq currents exist, the crochet in *H* was due to the increase in the Sq currents only. At the equatorial stations, Trivandrum, Kodaikanal and Annamalainagar, the crochet in *H* was due to the combined effects of the intensifications of the normal Sq currents as well as of the counter-electrojet currents at a slightly lower altitude. Thus the magnitude of crochet in *H* on May 3, 1973 increased with distance of the stations from the Equator.

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Accretional capture of the Moon

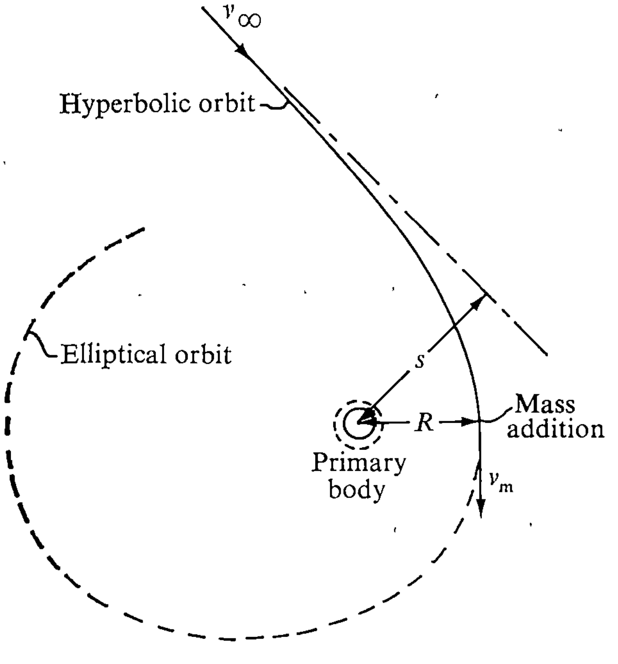
HYPOTHESES for the formation of the Earth–Moon system fall into one of three categories. The Moon was either (1) formed by fission from the Earth, (2) formed by accretion in Earth orbit, or (3) formed in solar orbit and then captured. We suggest that hypotheses (2) and (3) may be combined, with the result that many of the constraints on lunar origin are satisfied.

The primary difficulty with the hypothesis of lunar capture is its inherent improbability. Either collision or escape is much more likely, but capture could take place if sufficient dissipation occurred during a close approach of the Earth and Moon. It is unlikely, however, that tidal dissipation would be sufficient; an alternative would be collisional interactions with material already in Earth orbit[1]. Capture could also occur because of a change of mass of the Earth or Moon during a close approach[2]. It is this mechanism which we will discuss here. The closely related problem of orbital behaviour during mass loss from a binary star system has been considered by a number of authors[3–5].

Many calculations of planetary accretion have been carried out using particle mechanics. These calculations show that when an accreting body reaches a critical size, the gravitational attraction of the body greatly increases the rate of accretion. Opik[6] showed that if the Earth and Moon were accreted from common source material, and if the Earth was only slightly larger than the Moon during the early stages of accretion, the preferential gravitational accretion of the larger body could lead to the present mass difference. One consequence of this hypothesis is that the Moon should be enriched in early condensates; this is in fact the case[7,8]. We propose that capture took place during the final stages of the accretional process.

To estimate the effectiveness of accretional capture, we consider the following simple model: a small secondary body of mass *m*, in a hyperbolic orbit, approaches a large primary body of mass *M*. When the separation is a minimum, the mass of the primary body is increased to $M+\Delta M$. We derive the conditions under which this change in mass will result in an elliptic orbit (capture). The process is illustrated in Fig. 1.

Fig. 1 Illustration of accretional capture: the transfer from an initial hyperbolic orbit to an elliptical orbit due to mass addition of mass to the primary body at the point of closest approach.



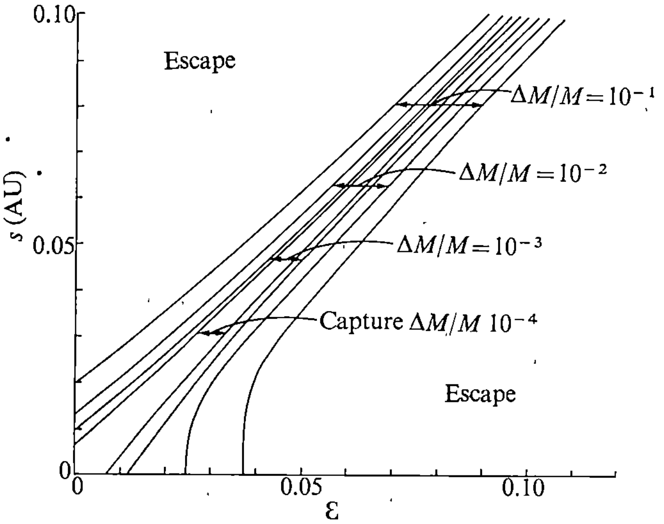


Fig. 2 Capture window for accretional capture: required values for the minimum separation s and eccentricity ε of the lunar orbit for various fractional increases in the Earth mass at the point of closest approach.

The minimum radial separation R, and the corresponding maximum relative velocity v_m are related to the approach velocity v_∞ and the impact parameter s by

$$\frac{s}{R} = \frac{v_m}{v_\infty} = \frac{GM}{s{v_\infty}^2} + \left(1 + \frac{G^2M^2}{s^2{v_\infty}^4}\right)^{1/2} \tag{1}$$

The sudden increase in the mass of the primary body is a non-conservative process, since there is a discontinuous change in the potential energy of the system. The secondary body suddenly finds itself in a stronger gravitational field, and may not have enough potential energy to escape.

After the change of mass, the total energy of the system is

$$E = \tfrac{1}{2}m{v_m}^2 - \frac{m(M+\Delta M)G}{R} \tag{2}$$

Capture occurs when $E<0$; the threshold value for capture is $E = 0$. Combining (1) and (2) with the condition $E = 0$ gives the mass increase required for capture

$$\Delta M = \frac{M}{2}\left[\left(1+\frac{s^2{v_\infty}^4}{M^2G^2}\right)^{1/2} - 1\right] \tag{3}$$

In order to relate the impact parameter s to the approach velocity v_∞, we assume that the two bodies are in independent orbits about the Sun. The primary body (the Earth) is assumed to be in a circular orbit with a radius r_e of 1 AU; the secondary body (the Moon) is assumed to be in an elliptic orbit with an eccentricity ε. The impact parameter s is taken to be the minimum radial separation between the bodies, and v_∞ the corresponding difference in orbital velocities; assuming $s/r_e \ll 1$ and $\varepsilon \sim 1$ these are related by

$$v_\infty = \pm\tfrac{1}{2}\left(\frac{GM_\odot}{r_e}\right)^{1/2}\left(\frac{s}{r_e} - \varepsilon\right) \tag{4}$$

Note that if $\varepsilon \approx s/r_e$, the approach velocity is small and the probability of capture is enhanced.

Combining equations (3) and (4) gives

$$\frac{s}{r_e} - \varepsilon = \left(64\frac{M\Delta M{r_e}^2}{{M_\odot}^2 s^2}\right)^{1/4} \tag{5}$$

Taking M to be the present mass of the Earth, the necessary conditions for capture are given in Fig. 2 for various values of $\Delta M/M$. It is seen that there is a well defined capture window even for small mass increments. This capture window corresponds to the condition of low relative velocity when $\varepsilon \approx s/r_e$.

Clearly the above analysis is highly idealised. The influences of a finite rate of mass addition and of the gravitational attraction of the Sun should be considered. The analysis does, however, indicate the possible importance of accretional capture, and indicates that further studies should be carried out.

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Petrogenesis of eucrite, howardite and diogenite meteorites

THE mineralogical, textural and compositional characteristics of the eucrite meteorites suggest that they are the products of igneous processes. Howardites are polymict breccias containing a variety of fragments, the bulk of which are thought to have crystallised from eucrite and eucrite-related melts[1]. Diogenites are orthopyroxene-rich stones which may represent cumulates from eucrite-related melts[2]. I relate here experimentally determined phase equilibria to the igneous processes which produced the eucrites and their associates.

I conducted melting experiments on a sample of the Juvinas eucrite (Harvard 87) at 1 atm total pressure and various oxygen fugacities. Samples were held in iron or molybdenum foil capsules in quenching furnaces with oxygen fugacity controlled by H_2–CO_2 gas mixtures[3]. Phase compositions were determined with an electron microprobe. At $f_{O_2} = 10^{-13.4}$ atmospheres and less than 10 °C below the metal-saturated olivine liquidus (Fo_{67}; 1,183 °C), the sample is saturated with olivine (Fo_{65}), pigeonite (Wo_5En_{65}), plagioclase (An_{94}), chrome-rich spinel and iron metal. On cooling, olivine reacts out by 1,150 °C and a silica polymorph crystallises from 1,085 °C.

Figure 1 is a projection of a low pressure liquidus diagram relevant to eucrite crystallisation. Olivine is in reaction relation with liquids at peritectic *A* (Fig. 1), and on the olivine + pyroxene boundary curve. Eucrite compositions have been projected onto this diagram. The clustering of non-cumulate eucrite compositions about the olivine + low calcium pyroxene + plagioclase + chrome spinel + metal peritectic (*A*, Fig. 1) indicates that like Juvinas, these other eucrites are probably multiply saturated near their liquidi. This emphasises the role of low pressure crystal–liquid equilibria in the genesis of the eucrites: all known eucrites can be designated as either multiply saturated liquids at *A* (Fig. 1) or cumulates or partial cumulates of the pyroxene and plagioclase near the liquidi of such liquids. Any model of the igneous processes which produced the eucrites must account for this preferential generation of liquids at peritectic *A* (Fig. 1).

The compositions of pyroxenes produced in the Juvinas experiments are similar to the compositions of pyroxenes in

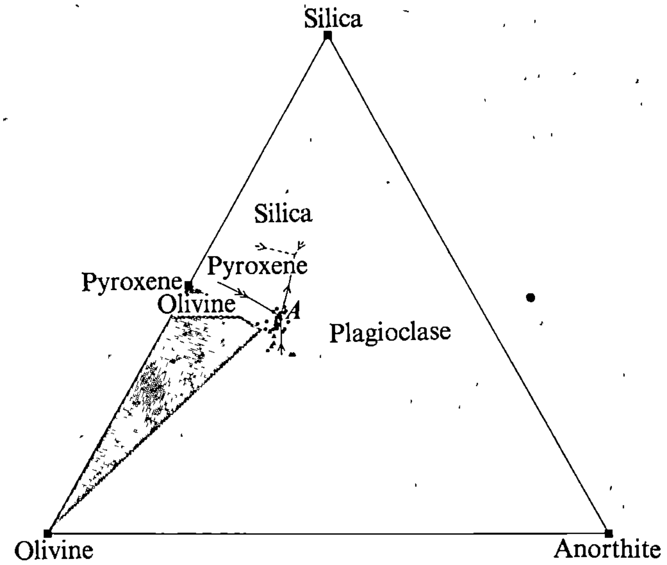

Fig. 1 Projection of low pressure liquidus diagram valid for molar Fe/Fe+Mg ≈ 0.60. Field boundaries were located by microprobe analyses of multiply saturated liquids from experiments on the Juvinas eucrite. Iron metal and chrome-rich spinel are present in all fields. ▲, Projected analyses of cumulate eucrites: Moore County (2 analyses), Serra de Magé (2 analyses), Moama, Medanitos; ●, Projected analyses of other eucrites. Pre-1960 analyses are plotted only when a more recent analysis of the same stone is not available. Analyses are plotted on this diagram by recalculating SiO_2, Al_2O_3, FeO, MgO, CaO, Na_2O and K_2O in terms of molar SiO_2, $CaAl_2Si_2O_8$, Fe_2SiO_4, Mg_2SiO_4, $CaSiO_3$, $NaAlSi_3O_8$ and $KAlSi_3O_8$ and then projecting on to the olivine–anorthite–silica plane. Analyses which plot below the pyroxene–plagioclase join do so because of the effects of modal iron metal, troilite, chromite, ilmenite and calcite on the projection. *A* and the shaded region, are explained in the text.

cumulate and non-cumulate textured eucrites and to those of many pyroxenes in howardites, but are more Fe-rich than the pyroxenes and olivines in diogenites and the most magnesian pyroxenes and olivines in howardites. These natural Mg-rich phases would have equilibrated with liquids more magnesian than the eucrite magmas at *A* (Fig. 1), and any model of eucrite petrogenesis should account for the existence of such liquids.

Reduction of eucrite magma resulting in crystallisation of iron metal and a decrease in the Fe/Mg and Fe/Mn of the magma could produce magmas which would crystallise magnesian diogenitic and howarditic pyroxenes. The pyroxenes crystallised from such liquids would have a higher Mn/Fe than those crystallised from unreduced eucrite magmas. Such an origin for diogenitic and the most magnesian howarditic pyroxenes is, however, unlikely since the Mn/Fe ratios of these phases are nearly identical to those of pyroxenes in cumulate and non-cumulate eucrites and of the more iron-rich howarditic pyroxenes[2,4].

Models which produce eucrite liquids by the differentiation of magnesian parental magmas and which invoke the fractional crystallisation of eucrite magmas to explain the bulk of the chemical variation observed in eucrites[2] encounter several difficulties. First, as pointed out in connection with Fra Mauro rocks and soils, which display a similar clustering about an olivine + plagioclase + low calcium pyroxene peritectic, it is difficult to preferentially generate liquids at peritectic *A* (Fig. 1) by low pressure differentiation of other magmas through the action of crystallisation processes[5]. Second, if the proposed parental magmas ever passed through a stage in their evolution in which pyroxene was the only crystallising phase (required by such models to produce the diogenites and magnesian pyroxenes in howardites), the liquid lines of descent of such magmas would never encounter the peritectic at *A* (Fig. 1). Third, low pressure fractional crystallisation paths which can produce liquids at *A* (Fig. 1) involve the crystallisation of plagioclase and olivine, but not of pyroxene. At low oxygen fugacities, the fractionation of plagioclase would rapidly differentiate europium from the other rare earth elements. Models which attempt to derive magmas at peritectic *A* (Fig. 1) by the low pressure fractional crystallisation of more magnesian magmas are, therefore, not easily reconciled with the primitive rare earth distributions of some eucrites[6], the existence of diogenitic and howarditic pyroxenes more magnesian than the pyroxenes at the liquidi of magmas at *A* (Fig. 1), or the rarity of olivine in diogenites, howardites and eucrites. Fourth, these models cannot easily reconcile the constancy of the major element chemistry (particularly the Fe/Fe+Mg ratios) of eucrites which cluster at *A* (Fig. 1) with their variations in rare earth and incompatible element concentrations[6]. Though the variations in the concentrations of rare earths and incompatible elements in the eucrites at *A* (Fig. 1) apparently suggest that these eucrites could be related to each other through the fractional crystallisation of up to 60% of a liquid of the composition of Haraiya[2] (another eucrite which projects at *A* (Fig. 1)), that suggestion is incompatible with the fact that all of these eucrites have nearly identical major element chemistries. A liquid which has reached *A* (Fig. 1) will not remain there during fractional crystallisation; as it loses heat it will change composition along the pyroxene + plagioclase cotectic and trend toward iron enrichment. Fifth, if these models are valid, the total volume of the magnesian parental magma must have greatly exceeded the volume of eucrite magma. Examples of magmas more primitive than the eucrites should predominate among falls and the distribution of fragments in howardites should be strongly skewed toward the products of magmas more magnesian than the eucrites at peritectic *A* (Fig. 1). These relationships are not observed.

I propose the following model to account for the observed features of the eucrites and their associates. The eucrite magmas at the peritectic *A* (Fig. 1) are interpreted as the products of small degrees of low pressure partial melting of an olivine + low calcium pyroxene + plagioclase + chrome spinel + iron metal source which projects in the shaded region of Fig. 1. Compositional variation of the eucrites is the result of variable degrees of partial melting and subsequent fractional crystallisation and crystal accumulation. Diogenites are regarded as cumulates of phases separated from more advanced partial melts of the same source. Howardites are polymict breccias containing fragments sampling the spectrum of differentiated liquids and cumulates developed from the range of primary magmas produced by variable degrees of partial melting of this source.

Eucrite magmas produced by the partial melting of such a source would plot at the peritectic *A* (Fig. 1) until plagioclase was exhausted from the source region; this explains the observed clustering of eucrite compositions. If the eucrites represent small degrees of partial melting, variations in the degrees of partial melting which produced different eucrite liquids could have produced the significant variations in the concentrations of incompatible elements which are observed between eucrites of indistinguishable major element chemistry and Fe/Fe+Mg ratios[6]. If plagioclase becomes exhausted from the source region at small degrees of partial melting, this model can also explain the variations in rare earth distributions and the magnitudes of europium anomalies which are observed between eucrites of nearly identical major element chemistry[6].

The subsequent crystallisation and separation of pyroxene and plagioclase from the primary magmas at *A* (Fig. 1) would be expected to produce cumulates and silica and incompatible element enriched liquids of higher Fe/Fe+Mg ratios projecting near the pyroxene + plagioclase cotectic emanating from *A* (Fig. 1). Moore County and Serra de Magé are examples of these cumulates[1]. The bulk chemistry and mineralogy of Binda, considered by some to represent a magma parental to the eucrites[2], are compatible with such a cumulate origin. Nuevo Laredo and

Lakangaon may represent liquid differentiates from the crystallisation of magmas at *A* (Fig. 1). Both are enriched in incompatible elements and display the iron enrichment expected in differentiated liquids. It is unlikely that they represent smaller degrees of partial melting of the same source that produced the eucrites which are not iron enriched, for at least in the case of Nuevo Laredo, rare earth distributions are not compatible with this interpretation[6]. Other eucrites may have been affected to a lesser extent by fractional crystallisation.

Though olivine would be present in the source rock and the residue left after melting, it would not be present at the liquidi of the partial melts at *A* (Fig. 1), (provided there was little fall in pressure accompanying eruption) because it is in reaction relation with these liquids. This is consistent with the absence of olivine in eucrites and its rarity in howardites.

If the reaction relationship between iron metal and silicate liquids observed in synthetic systems[7] extends to this natural system, metal would remain in the source region until all crystalline silicate phases were removed by melting. The probable reaction relation between chrome-rich spinel, low calcium pyroxene and liquid[8] suggests that chrome spinel would remain in the source region until all the pyroxene had been removed by melting.

In conditions of fractional fusion[9], the production of magma by further melting would be nearly halted after the exhaustion of plagioclase from a source projecting in the shaded region of Fig. 1. If equilibrium fusion or imperfect fractional fusion took place, the melts produced after the exhaustion of plagioclase from the source would move up the olivine + pyroxene curve (Fig. 1), continuously decreasing the Fe/Fe+Mg ratio, until pyroxene was exhausted from the source. The diogenites and magnesian pyroxenes in howardites can be interpreted as cumulates of the pyroxene crystallising near the liquidi of these more advanced partial melts. The rarity of olivine in these meteorites is predicted since these liquids would not ordinarily crystallise olivine. The occasional presence of olivine in these meteorites (and Chassigny) could result from variations in source composition, variations in conditions of melting or crystallisation (for example, a small drop in pressure between the source region and the environment of crystallisation), or xenocrysts from the imperfect separation of the magma from the source. The absence of cumulate plagioclase in the diogenites or in intergrowths with the most magnesian howarditic pyroxenes[4] can be expected since plagioclase would not be present at the liquidi of these magmas.

The residue left in the source region after the exhaustion of plagioclase by partial melting would be olivine + low calcium pyroxene + chrome spinel + metal. If metal were a major constituent of the source, this residue would approximate the Lodran meteorite[10]. If the source were low in metal, the olivine-bearing diogenites could represent such residues. Since such an origin is not possible for the olivine-free diogenites, a cumulate origin is preferred for the entire diogenite group.

Further equilibrium fusion or imperfect fractional fusion of this same source after the exhaustion of pyroxene from the source region would produce liquids moving away from the olivine + pyroxene reaction curve (Fig. 1) towards the olivine composition, leaving a pallasite-like residue of olivine + chrome spinel + metal. The olivines crystallised near the liquidi of such melts may account for some howarditic and diogenitic olivines.

The abundance patterns of fragments in howardites[4] and the lack of known meteorites with compositions which could represent partial melts more advanced than the eucrites at *A* (Fig. 1) are consistent with the bias toward the products of smaller degrees of partial melting which is expected in conditions of imperfect fractional fusion.

Though eucrite liquids would have equilibrated with the metal of the residue left in the source region after partial melting, metal would not be at the low pressure liquidi of these melts because of the probable reaction relation between metal and silicate liquid (provided no change in oxidation state of the magmas occurred between the source region and the environment of crystallisation). This is compatible with both the observed low Ni contents of the eucrites, which suggest they have equilibrated with metal, and the petrographic observations that metal is never found as a cumulate phase in diogenites and cumulate eucrites, is rarely enclosed in pyroxenes, and is usually a late, interstitial phase when it occurs[11]. The late stage crystallisation of metal in eucrites could be attributable to desulphurisation processes similar to those which have been proposed to account for metal in lunar mare basalts[12].

A partial melting model requires a suitable low pressure source assemblage in the eucrite parent body. A primitive type of mesosiderite (perhaps a condensate[13] or the product of early differentiation and brecciation on the eucrite planet or on some other body which was subsequently incorporated into the eucrite planet) could be suitable source material. The low pressure mineralogy (< 8 kbar) of the bulk composition of the eucrite parent body calculated by Wänke and Palme[14] is similar to the source assemblage required by this model. This similarity raises the possibility that the eucrites may have formed by partial melting of the primitive material of the eucrite parent body at 4.6×10^9 yr BP.

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Does metallic ammonium exist?

THE ammonium ion NH_4^+ behaves in many ways like an alkali metal ion. This led Ramsey[1] to propose that monovalent ammonium metal (NH_4^+ ions immersed in an almost uniform, degenerate sea of electrons) is stable at pressures much lower than typical insulator–metal transition pressures[2] of megabars $\sim 10^{11}$ Pa (10^5 Pa = 1 bar). Subsequently, it was estimated[3] that the transition from a mixture of NH_3 and H_2 molecules to metallic ammonium, occurs at a pressure of less than 2.5×10^{10} Pa. If this estimate were correct, metallic ammonium would be of great interest to experimental high pressure physicists, and to planetary physicists. Indeed, metallic ammonium has been invoked in some[4,5] but not all models for the interiors of Uranus and Neptune; and its existence could be of importance[6] for calculations of the interior magnetic fields of these planets.

Here I report new calculations which demonstrate, rather conclusively, that metallic ammonium, at least in the form envisaged by Ramsey, is unstable at all pressures. My calculations disagree with those of ref. 3 in two important respects: first, I find that the insulating NH_3–$\frac{1}{2}H_2$ mixture is sufficiently compressible to remain the favoured phase, at least until pressures of $\sim 10^{11}$ Pa, even if the metallic phase calculation of ref. 3 is correct. Second, my calculation for the metallic phase gives a systematically higher energy than was obtained in ref. 3. These results are summarised in Fig. 1.

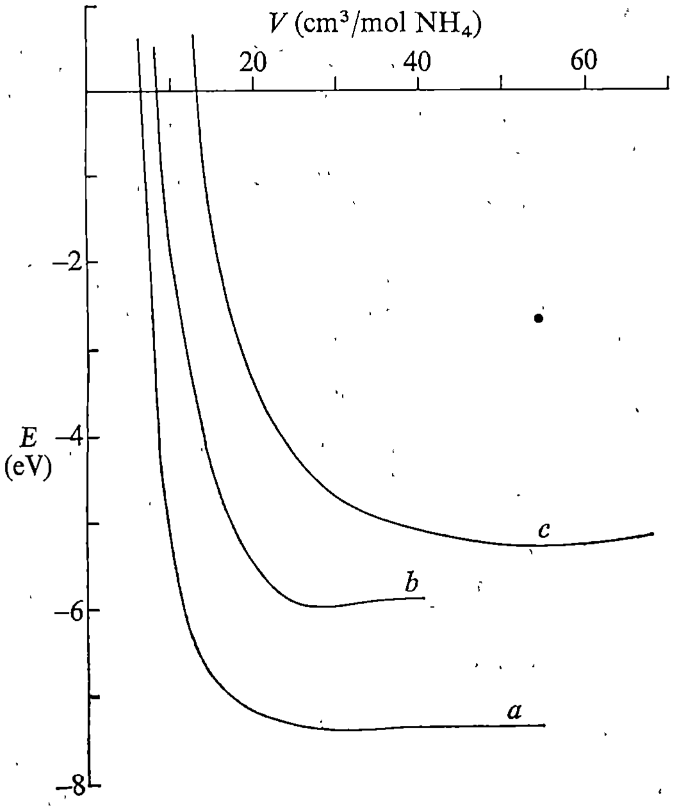

Fig. 1 A comparison of the energy–volume relationships for molecular NH_3–$\frac{1}{2}H_2$ (*a*) and metallic ammonium (*b*, ref. 3; *c*, my results).

The equation of state for the NH_3–$\frac{1}{2}H_2$ mixture was calculated assuming volume additivity, so that $V(P)$, the specific volume of the mixture at pressure P, is given by

$$V(P) = V(NH_3,P)+\tfrac{1}{2}V(H_2,P) \qquad (1)$$

where $V(NH_3,P)$ and $V(H_2,P)$ are the molecular volume–pressure relations of ammonia and hydrogen solids, respectively. The equation of state for ammonia is known at low pressures ($P < 2\times10^9$ Pa) from experiment[7], and at very high pressures ($P \gtrsim 2\times10^{11}$ Pa) from Thomas–Fermi–Dirac theory[8]. The intermediate region is estimated by interpolation, but the uncertainties in volume do not exceed $\sim$ 15%. The equation of state for hydrogen is taken from Ross's analysis[9] of the Livermore shock data. From $V(P)$, the energy of the mixture is evaluated:

$$E(P)-E(0) = -\int_0^P P'\frac{dV(P')}{dP'}dP' \qquad (2)$$

at $T = 0$ °K. In Fig. 1, the energy zero is chosen to be the energy of infinitely dispersed NH_4^+ ions and electrons. Thus,

$$E(0) = -(\tfrac{1}{2}D+I_H-A_p+B) \qquad (3)$$

where $D = 4.44$ eV is the dissociation energy of the hydrogen molecule, $I_H = 13.54$ eV is the ionisation energy of the hydrogen atom, A_p is the proton affinity of the ammonia molecule, and $B \sim 0.1$ or 0.2 eV is the binding energy of the NH_3–$\frac{1}{2}H_2$ crystal mixture. The most likely value of A_p is 8.6 eV (ref. 10), but the error in this figure could be as much as 0.3 eV. This gives $E(0) = -7.3$ eV. The resulting energy–volume relation is shown in Fig. 1. Even allowing for the uncertainties in A_p and $V(P)$, the curve never crosses the metallic phase curve of ref. 3. Their transition pressure estimate is invalid since they did not calculate the NH_3–$\frac{1}{2}H_2$ equation of state, but merely constructed that tangent to the metal-phase curve which passes through the molecular-phase zero pressure point.

It must be emphasised that my conclusion is not contingent on the validity of volume additivity (equation (1)). My calculation is correct for a conceivable state: a mixture of macroscopic crystals of pure NH_3 and H_2. If an alloy is preferentially formed it can only have lower energy than the calculation predicts—this would make the molecular phase even more favourable.

Since the NH_4^+ ion is about the same size as the Rb^+ ion, simple pseudopotential theory[11] would lead us to expect that the equilibrium ($P = 0$) volume per ion of metallic ammonium is comparable with that of rubidium. The detailed calculations confirm this prediction of the simple (but very successful) pseudopotential theory. In contrast, an equilibrium volume was found in ref. 3 that was about a factor of two smaller, and more comparable to metallic sodium. My calculation is based on the Wigner–Seitz approach, but with the potential given by the self-consistent density-functional theory of Kohn and Sham[12], which includes all exchange and correlation effects in a local but self-consistent approximation. The Bardeen perturbation theory[13] was used to evaluate the effective (band) mass of the conduction electrons.

The method was tested on metallic sodium, and the results were −6.38 eV for the total energy per ion, and $3.96a_0$ for the radius of the Wigner–Seitz sphere (where $a_0 = 1$ Bohr $= 0.529\times10^{-8}$ cm); these are in excellent agreement with the observed values[12] of −6.27 eV and $3.93a_0$, respectively. For metallic ammonium, I used the spherical potential of ref. 3 and also their core wavefunctions. The results were −5.40 eV for the energy and $5.18a_0$ for the radius, whereas they obtained −5.84 eV and $4.23a_0$, respectively. I also made a calculation using more accurate core wavefunctions[14] and a correspondingly larger N–H bond length ($1.91a_0$ instead of $1.84a_0$), obtaining −5.36 eV and $5.35a_0$ for $P = 0$. The corresponding energy–volume relation is the upper curve in Fig. 1. At high pressure, the energy probably rises even more rapidly than shown, because of the Born–Mayer repulsion between the ion cores. Corrections for the non-spherical symmetry of the NH_4^+ ion have been estimated and are negligible at low pressures.

I conclude that monovalent metallic ammonium is never the thermodynamically favoured phase for an NH_3–$\frac{1}{2}H_2$ mixture. Of course, this mixture must eventually become metallic if compressed enough, but this would not occur until pressures of $\sim 10^{11}$ Pa. The very compact metallic phase that would then form is unrelated to the low density metallic phase proposed by Ramsey.

I notice, finally, that a dynamo-generated magnetic field in Uranus or Neptune is not contingent on the existence of metallic ammonium—other sources of electrical conductivity may be adequate (this will be dealt with elsewhere).

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Mercury in the Kuroshio and Oyashio regions and the Japan Sea

ALTHOUGH measurements of the mercury concentration in sea water have been made by several investigators[1–13], the reported values are widely scattered and it may be significant that almost all the samples were kept in polyethylene bottles after acidification. Weiss *et al.*[14] have reported that the main source of mercury in the oceans was the degassing of the Earth's crust by way of the atmosphere. It should then be supposed that the large experimental variations for open oceans does not reflect the true situation.

One source of error is to be found in the use of polyethylene bottles—we found that nanogram amounts of mercury were leached out of the plastic by the samples, whose mercury concentrations increased by factors of 2 to 100, even if the bottles had been washed out with nitric or hydrochloric acid before use. The outline has already been reported[15], and the detailed results will be published elsewhere. Recently, sample contamination by polyethylene bottles has been confirmed[16,17]. In our work, glass bottles and stoppers, which had been checked to be non-contaminating beforehand, were used for sample storage.

Table 1 Mercury in the Kuroshio and Oyashio regions and the Japan Sea

Station Date of sampling	Depth (m)	Temperature (°C)	Salinity (‰)	Hg (ng l^{-1})
1 42° 30″ N 144° 00″ E February 1974	0	0.2	32.68	4.5
	20	0.18	32.68	5.6
	49	0.80	32.86	5.6
	98	0 85	32.94	4.6
	196	1.38	33.16	4.6
2 37° 00″ N 114° 00″ E February 1974	0	3.00	33.12	5.4
	21	3.52	33.30	3.9
	52	3.53	33.33	4.7
	101	2.46	33.30	5.5
	200	2.45	33.44	5.5
	500	3.73	34.07	5.6
	1,009	2.90	34.40	5.5
	1,219	2.58	34.46	5.2
3 36° 56″ N 141° 58″ E February 1974	0	15.6	34.74	5.4
	20	15.81	34.75	5.2
	50	15.78	34.74	5.4
	101	15.56	34.72	4.9
	197	12.85	34.50	5.4
	486	4.24	33.87	5 4
	782	3.37	34.12	4.9
	1,200	2 65	34.43	5.0
4 35° 01″ N 142° 04″ E March 1974	0	18.4	34.83	5.2
	21	18.50	34.81	5.4
	52	18.51	34.81	4 5
	102	18.48	34.80	5.0
	247	16.59	34.69	4.5
	557	8.49	34.22	4.8
	1,036	3 72	34.37	4.9
	1,233	3.13	34.41	5.0
5 34° 00″ N 142° 00″ E March 1974	0	17.2	34.78	5.0
	50	17 13	34.79	4.0
	102	17.09	34.79	3.6
6 33° 11″ N 127° 32″ E August 1974	0	28.0	32.73	5.1
	17	26.99	33.39	5.3
	100	15.81	34 43	4.9
7 33° 23″ N 128° 35″ E August 1974	0	27.9	33.42	5.4
	9	27.58	33.40	5.6
	70	22.39	34.25	5.4
	94	20.48	34.25	5.4
8 38° 41″ N 135° 55″ E July 1974	0	22.2	33.50	4.3
	20	18.87	33.72	4.3
	102	13.05	34.44	5.4
	202	3.57	34.09	4.8
	400	0.56	34.07	4.3
	697	0.19	34.07	4.8
9 39° 17″ N 135° 55″ E July 1974	0	20.9	33.71	5 0
	20	18.50	33.85	5.2
	50	11.01	34.34	5.0
	150	2.54	34.08	4.3
	200	1.37	34.06	5.6
	397	0.40	34 02	4.5
	695	0.20	34.01	5.4

Here we report on the mercury concentrations in the Kuroshio and Oyashio regions and the Japan Sea. Samples were collected with Nansen bottles from February to August 1974. Immediately after collection, the water was acidified to 0.4 N with sulphuric acid, and stored in a glass bottle. The samples were analysed at the laboratory after a month's storage.

The outline of the analytical method is as follows[18]. Ionic mercury, in a 0.5–0.8-l sample solution, is reduced with stannous chloride, and the mercury vapour released is flushed out with nitrogen on to gold particles packed in a glass tube. The metal is then heated at 500 °C in a furnace, and the vaporised mercury is determined using an atomic absorption spectrophotometer. The error in a single determination was ~ 10% when measuring quantities of ~ 5 ng Hg.

The results are shown in Table 1. Although there were haloclines and thermoclines in the seas at our sampling points, no appreciable change of mercury concentration with depth was found, and the samples showed an almost constant value, 5.0 ± 0.5 ng l^{-1}.

According to Fitzgerald *et al.*[12], if the seawater samples had been stored in plastic bottles, at *p*H 1, for 10 days, the mercury concentrations would have been 57% lower than those in samples measured immediately, which they therefore did—on board their ship.

We found, however, that for sea water of more than 30‰ salinity, the mercury concentrations were 30% higher than in the samples measured immediately. We believe that the discrepancy arises because of the presence of some mercuric complexes, whose mercury cannot be detected immediately. Acidification to 0.4 N with sulphuric acid for two weeks at room temperature (and longer at lower temperatures) is required to release this mercury, and give the correct total mercury concentration. (For samples with salinities of less than 30‰, the mercury concentration actually decreases, because of adsorption to the glass walls.)

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Model for the accumulation of radionuclides in oysters and sediments

THE time variation of the concentration of γ-ray emitting radionuclides has been measured and modelled in a stable population of American oysters (*C. virginica*), and associated estuarine sediments. Radionuclide concentrations were measured for the natural radionuclides ^{212}Pb and ^{40}K and for the man-made radionuclides ^{58}Co, ^{60}Co, ^{54}Mn, ^{95}Nb, ^{134}Cs and ^{137}Cs using a Ge(Li) detector with a massive lead shield and multichannel analyser. Measured concentrations of the most abundant man-made radionuclides observed in this study (^{58}Co, ^{60}Co, and ^{54}Mn) were compared with concentrations calculated with a mathematical model for the accumulation and loss of these radionuclides by oysters and sediments.

Much work has been done in recent years in the accumulation of radionuclides in marine organisms (refs 1 to 4, and references therein). Mathematical models for accumulation and loss of radionuclides are usually based on laboratory studies using a constant source of radionuclides, a condition which is generally convenient. A model for field studies, however, must provide for the time variation of the source of radionuclides, since in the case of nuclear reactors the liquid radionuclide effluent is usually released according to some schedule with various waiting periods between releases, as discussed by Heft *et al.*[2]. This time variation of source intensity can be included in the mathematical model by driving the first order linear differential equation for accumulation and loss with a time varying function representing the release schedule. This model corresponds mathematically to a driven system with characteristic relaxation times for the various mechanisms of accumulation and loss.

American oysters *C. virginica* were reared at four locations at the Montsweag Estuary of the Sheepscot River in southern-central coastal Maine, USA and at a control site six miles east on the Damariscotta River Estuary.

At two month intervals, ~1 kg of live oysters from each site were randomly selected from a larger population of the same age and size. Each sample was removed from its rearing tray, transported to the detector, γ-ray counted in a standard geometry for 5,000 s, and returned to its site. The resulting counts were computer processed using the Compton continuum subtraction method[5]. The concentrations of radionuclides were determined using standard techniques to calibrate the multichannel analyser–detector–shield system.

Sediments were collected at the same five sites by removing the first centimetre of sediment over an area of sufficient size to provide a 2 kg (wet) sample. Concentrations of radionuclides in the sediments were measured in the same manner as the oysters.

The uptake of radionuclides may be described by a first order linear differential equation

$$\mathrm{d}N/\mathrm{d}t = -\lambda N + R(t), \tag{1}$$

where $\mathrm{d}N/\mathrm{d}t$ is the change in atoms of radionuclide, λN is the rate of loss due to radioactive decay, and $R(t)$ is the rate of introduction of radionuclide from an external source (the nuclear reactor release schedule)[6]. Depuration, or biological loss, may be included by writing a 'lambda effective' as the sum of the radioactive decay constant and a biological decay constant.

The solution to equation (1) may be written

$$N = \exp(-\lambda t)\int^{t} R(\tau)\exp(\lambda\tau)\mathrm{d}\tau + c\exp(-\lambda t) \tag{2}$$

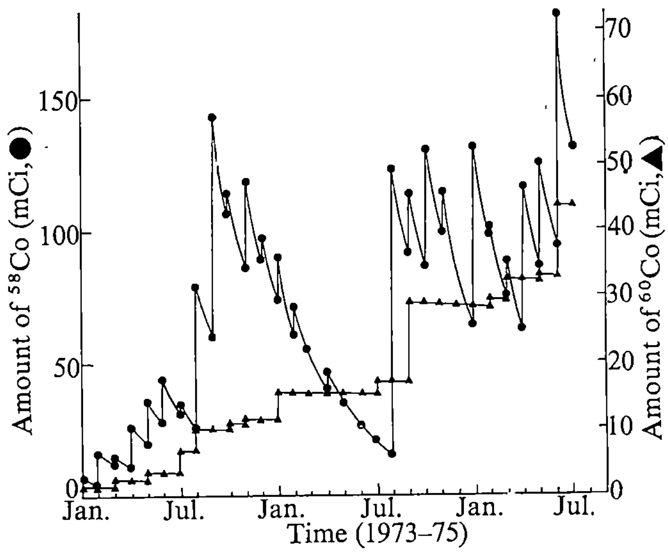


Fig. 1 ●, Theoretical accumulation of ^{58}Co in mCi for the case $U = 1\ \mathrm{g}^{-1}$ plotted against month of year from February 1973 through June 1975. ▲, Theoretical accumulation of ^{60}Co in mCi for the case $U = 1\ \mathrm{g}^{-1}$ against month of the year from February 1973 through June 1975.

We assume that releases of radionuclides are made in a sequence of m times, $\{t_1, t_2, t_3, \ldots .t_m\}$ and the amount of nuclide released is given by a function $f(t)$ where

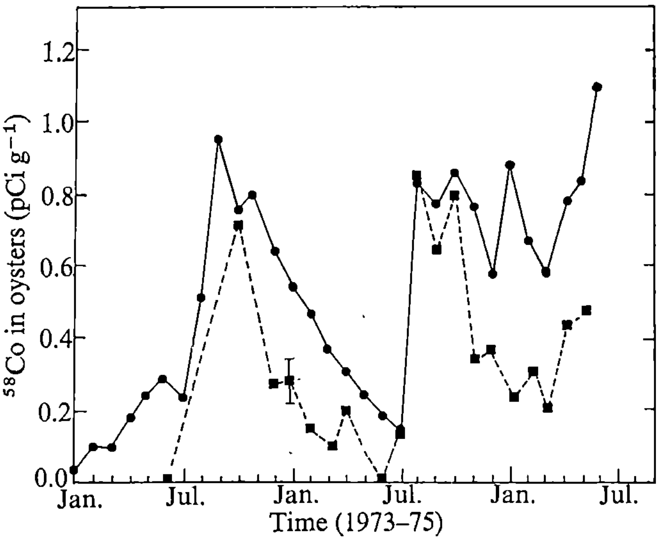
$$f(t) = f_1\delta(t-t_1) + f_2\delta(t-t_2) + \ldots . f_m\delta(t-t_m)$$

The amount of radionuclide release by the reactor and retained by the sample is given by U, so that, assuming U is a constant, we can construct a solution for the intervals between the times of release.

$$\begin{array}{ll} 0 \le t \le t_1 & N(t) = c\mathrm{e}^{-\lambda t} \\ t_1 \le t \le t_2 & N(t) = Uf_1\mathrm{e}^{-\lambda(t-t_1)} + c\mathrm{e}^{-\lambda t} \\ t_2 \le t \le t_3 & N(t) = Uf_2\mathrm{e}^{-\lambda(t-t_2)} + Uf_1\mathrm{e}^{-\lambda(t-t_1)} + c\mathrm{e}^{-\lambda t} \\ \cdot & \cdot \\ \cdot & \cdot \\ \cdot & \cdot \end{array} \tag{3}$$

The interpretation of this set of equations is obvious. Sudden increases occur at each time of release. Typically, the radionu-

Fig. 2 ■, Measured ^{58}Co radionuclide concentration in oysters at the reactor outflow site. A typical 1σ error bar is shown. ●, Theoretical curve for ^{58}Co scaled by factor $U = 7.6\times10^{-12}\ \mathrm{g}^{-1}$ against time in months for the years 1973–1975.



clides are released weekly, over a period of a few hours. For the purposes of illustrating the theory presented here, however, it has been found sufficient, for a bi-monthly sampling schedule, to approximate the actual release schedule with a monthly release in which the total amount of each radionuclide released is assumed to be released instantaneously. Graphs of two typical cases are shown in Fig. 1, which illustrates the results for ^{58}Co (half life 2.4 months) and ^{60}Co (half life 63 months).

The radionuclide concentration for ^{58}Co in oysters expressed in pCi g^{-1} is shown in Fig. 2. The broken line represents the experimental values for this radionuclide at the reactor outflow site and the theoretical results are shown with a solid line. The peaks found in the theory are evident at the reactor outflow as well as the other three Montsweag Estuary sites. In general it is shown that the oysters exhibit a faster decrease in activity than predicted by the radioactive decay constant only. An effective decay constant taking into account the biological decay constant as well can bring the theory into better agreement with experiment.

The concentration of the radionuclide ^{54}Mn for the reactor outflow site was found to be practically constant with time and compares favourably with the theoretical prediction. In addition to ^{58}Co and ^{54}Mn small amounts of ^{60}Co were observed, while the amount of naturally occurring radionuclide ^{40}K varied in time with the salinity at each site.

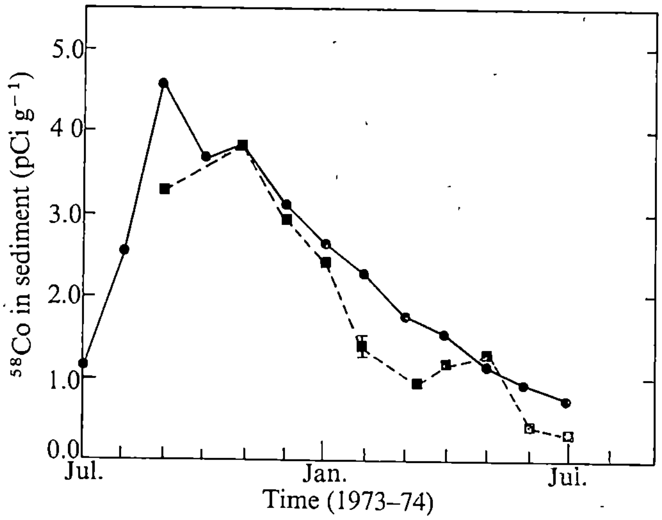


Fig. 3 ■, Measured ^{58}Co radionuclide concentration in sediment at the reactor outflow site. A typical 1σ error bar is shown.. ●, Theoretical curve for ^{58}Co scaled by factor $U = 31 \times 10^{-12}$ g^{-1} against time in months for the years 1973–1974.

The concentration of the radionuclide ^{58}Co in sediments is shown in Fig. 3. The broken lines represent the experimental values for this radionuclide and the solid line represents the theory. Again the decrease of ^{58}Co is more rapid than theory predicts, and in this case represents loss of radioactivity from the sediments by primarily physicochemical transport. The other three estuary sites had a factor of 10 less radionuclide than this particular site, but showed very similar variations with time.

Experimental measurements of sediments continued monthly after July 1974. These results are not, however, comparable with theory after this time because extensive construction in the estuary has temporarily changed and complicated the sedimentation.

For the sediments site at the outflow we observe the natural radionuclides ^{40}K and ^{212}Pb having average concentrations 5.7 pCi g^{-1} and 0.4 pCi g^{-1} respectively. We also observe the man-made radionuclides ^{58}Co, ^{60}Co, ^{54}Mn, ^{137}Cs, ^{134}Cs and ^{95}Nb with average concentrations of 4.9, 0.8, 0.2, 0.2, 0.4 and 0.8 pCi g^{-1}, respectively. These concentrations are presented to give a comparison of the natural and man-made radionuclides observed.

Since the theoretical and observed time dependence factors seem to agree, it is possible to divide the sample concentration (pCi g^{-1}) at each site by the release of radionuclides from the reactor (Ci) to form a measured uptake ratio U. The U values for ^{58}Co in oysters at the different sites in order of increasing distance from the outflow were 7.6, 5.6, 2.6 and 1.3, where the units of U are $g^{-1} \times 10^{-12}$. These U values indicate most of the ^{58}Co is concentrated in the region of the outflow site decreasing rapidly with distance from the reactor. The U values of the other isotopes (^{60}Co and ^{54}Mn) show the same trend, both in the oysters and sediments. A first principles calculation of U values, which has not been done, must take into consideration the reactor radionuclide effluent inventory; the concentration factors for the uptake medium, and the physical oceanography[7] of the estuary.

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Disordered drinking after abdominal vagotomy in rats

WHILE investigating the effect of abdominal vagotomy on satiety elicited by cholecystokinin[1], we noted that many vagotomised rats seemed to be undernourished and failed to regain their preoperative weight. Because of their low water–food ratio (vagotomised 1.3±0.5 ml g^{-1}, sham 1.8±0.1 ml g^{-1}, $P < 0.001$), we analysed their drinking behaviour in various conditions. We report here that abdominal vagotomy produces severe deficits in drinking behaviour that can be characterised as motivational.

Bilateral vagotomy was performed along the lower oesophagus in nine male rats (430–560 g) under Equithesin (Jensen–Salsbery Laboratories) anaesthesia. Sham vagotomy (manipulation of the oesophagus without nerve transection) was carried out in ten rats. Vagotomy was verified anatomically at the conclusion of the drinking tests without knowledge of the behavioural results. All nine vagotomised rats were completely disconnected.

Rats were housed individually, maintained on a 12-h light–dark cycle with continuous access to Purina pellets and tapwater except during drinking tests, when food was removed. Food and water intakes were measured daily for 1 week before and for 2 months after surgery. Drinking tests began 1 month after surgery when daily food and water intakes had stabilised.

Vagotomised rats drank much less water than sham rats during 24 h when no food was available (vagotomised 1.8±0.4 ml per 100 g body weight (BW), sham 6.1±0.8 ml per 100 g BW, $P < 0.001$; control data, Fig. 1). After 24 h water deprivation (with food available), vagotomised rats drank as much in 2 h as sham rats (vagotomised 2.4±0.3 ml per 100 g BW, sham 2.9±0.1 ml per 100 g BW) although five of the nine drank less than the smallest intake of sham rats. Vagotomised rats did not drink normally (Fig. 1) in the 2 h after cellular dehydration was produced by hypertonic saline

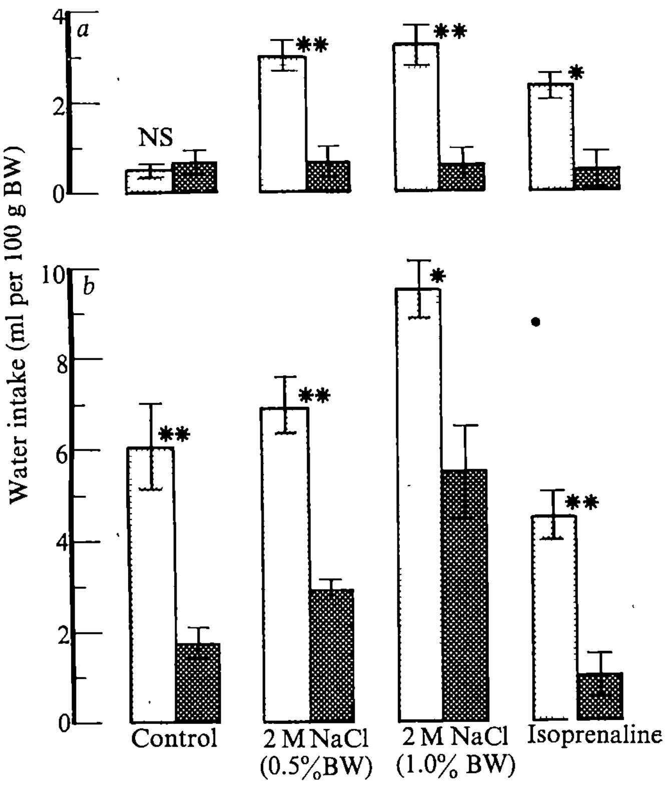

Fig. 1 Water intake (± s.e.) for sham (light bars) and vagotomised (dark bars) rats in 2 h (*a*) and 24 h (*b*) after NaCl or isoprenaline (0.16 mg per kg BW). There were at least eight rats in each group in control and NaCl conditions. There were four vagotomised and five sham-operated rats treated with isoprenaline. Variance is expressed as s.e. All statistical comparisons were made with a two-tailed *t*-test. NS, Not significantly different; * $P < 0.01$; ** $P < 0.001$.

(2 M NaCl, 0.5 or 1.0 ml per 100 g BW, intraperitoneally). The deficit was severe: two of the nine did not drink after 0.5% BW NaCl (0.5 ml per 100 g BW) and five did not drink after 1.0% BW NaCl (1.0 ml per 100 g BW). None of the lesioned rats seemed depressed by the saline load.

Four hours after salt loading, vagotomised rats still drank less (unpublished data), but by 24 h their intake above control was equal to that of sham rats (Fig. 1). These 24-h intakes should not be interpreted as drinking in response to intracellular dehydration alone, because urinary excretion of solute load probably also led to extracellular dehydration.

In response to isoprenaline[2], vagotomised rats also failed to drink normally: three did not drink and the other two drank less and had longer latencies than sham rats (Fig. 1).

Polyethylene glycol also elicits drinking through extracellular[3] and possibly intracellular[4] stimuli. Five vagotomised and four sham-operated rats were injected with polyethylene glycol (5 ml of 30% w/w solution in 0.9% NaCl, subcutaneously) and immediately offered water. After 8 h, three of five vagotomised rats had not drunk. By 24 h, however, all rats had drunk, but group intakes differed significantly (vagotomised 12±2.6 ml, sham 22±2.0 ml, $P < 0.02$). When NaCl (0.9%) was offered to drink 24 h after injection of polyethylene glycol, all rats drank large volumes in the 1-h test (vagotomised 38.4±9.2 ml, sham 34.6±5.7 ml). The normal intake of saline and the low intake of water after polyethylene glycol demonstrates that the effect of vagotomy on water intake cannot be due to painful or uncoordinated oesophageal movements.

In summary, bilateral abdominal vagotomy abolished the drinking response to acute cellular dehydration and to isoprenaline, decreased drinking in the absence of food and after polyethylene glycol, decreased the water–food ratio, and tended to decrease drinking after water deprivation. Transection of both vagal trunks seems to be necessary for these deficits, because drinking was normal in 2 rats with a large vagal connection persisting between oesophagus and stomach (unpublished). The critical region of vagal innervation that must be removed to obtain the drinking deficits was not determined, but the level of section preserved the vagal innervation of the liver and portal bed, which should have permitted normal function of postulated hepatic osmoreceptors[5].

Such experiments have not been reported before in rats, but the effect of vagotomy on thirst has been studied extensively in the dog. The results are different and seem to depend on the level of vagal section: abdominal vagotomy increased the size of a bout of drinking, but did not alter the 24-h water–food ratio[6]; thoracic vagotomy did not change the drinking response to hypertonic saline[7], but cervical vagosympathectomy enhanced drinking to hypertonic saline[8] and blocked the ability of extracellular dehydration (haemorrhage) to decrease the osmotic threshold for drinking to hypertonic saline[8]. This last effect is consistent with the decreased responses to the extracellular challenges of isoprenaline and polyethylene glycol reported here, but none of this work in the dog predicts the failure of drinking after hypertonic saline that we found.

It is not likely that these drinking deficits result from the removal of efferent vagal influence, because peripheral anticholinergic block produced by methylatropine or methylscopolamine does not diminish drinking after hypertonic saline[9,10] or water deprivation[11]. Furthermore, the peripheral and central anticholinergic effects of atropine do not block drinking after isoprenaline[2].

If peripheral anticholinergic block mimics all the effects of removal of vagal efferent function, then the failure of anticholinergics to reproduce the effects of abdominal vagotomy on drinking suggests that the critical lesion is the loss of vagal afferent stimuli. The role of vagal afferent stimuli from the abdominal viscera in mediating thirst is not known. Fitzsimons[12] has suggested the importance of vagal afferent stimuli, originating in the walls of the great vessels and atria, for eliciting thirst after extracellular dehydration. These regions, however, remained innervated in our vagotomised rats.

Although we do not know if vagotomy abolished pancreatic osmoreceptor[13] or mesenteric vascular volumetric stimulation, the fact that vagotomy abolished or attentuated drinking to a variety of stimuli suggests a deficiency in the motivation for drinking. The clearest example of the decreased responsiveness which characterises this lack of motivation is the drinking behaviour of vagotomised rats during 24 h when food was not present: they drank one-third as much as sham rats (Fig. 1).

In these experiments, the motivational deficit seemed to be restricted to drinking, because the 24-h food intake of vagotomised rats in the absence of water was not different from that of sham rats (vagotomised 3.8±0.3 g per 100 g BW, sham 3.7±0.1 g per 100 g BW). These data are not conclusive, however, and the specificity of the motivational deficit requires more rigorous testing.

Ball reported previously a motivational defect after abdominal vagotomy[14]. He observed elevated thresholds for lateral hypothalamic stimulus-bound eating and self stimulation in vagotomised rats. Ball suggested that this decreased excitability of the lateral hypothalamus after abdominal vagotomy was evidence for the importance of a vago–vagal, positive feedback loop in the mediation of these stimulus-bound behaviour patterns. Our interpretation of the results with drinking behaviour is analogous to Ball's suggestion for feeding and self stimulation.

If the disordered drinking observed after vagotomy were the result of elevated lateral hypothalamic thresholds, lateral hypothalamic damage should produce a similar deficit in drinking. This is confirmed by Stricker[15], who reported that rats recovered from lateral hypothalamic lesions did not drink after polyethylene glycol, ligation of the inferior vena cava or hypertonic saline during relatively brief (1–6 h) testing conditions, but did drink significant amounts of water by 24 h after treatment.

Although the drinking deficits after abdominal vagotomy and lateral hypothalamic lesions seem similar, it remains to be determined if this behavioural similarity signifies an important neurological relationship between afferent vagal stimuli from abdominal viscera and lateral hypothalamic integration of drinking behaviour.

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Fast body turns in a cichlid fish

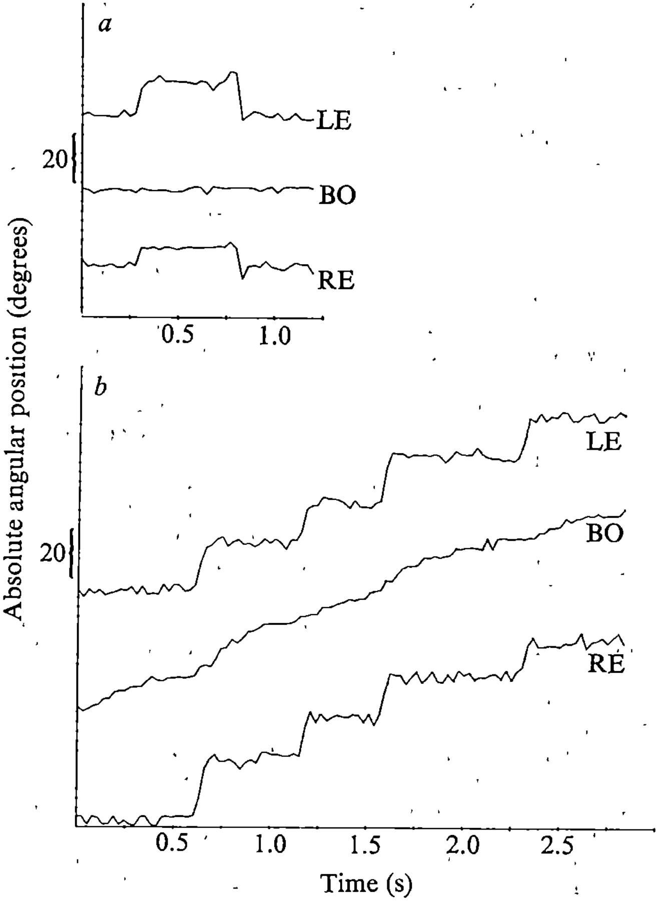
The African cichlid fish *Haplochromis burtoni*, is sexually dimorphic. The female is smaller and of an homogeneous beige colour, whereas the male has bright colour patterns which he can turn on and off quickly. These are important signals, particularly during aggressive interactions[1], since these fish rely extensively, although not exclusively, on visual cues in their social behaviour[2]. Sound production does not seem to be involved[3], but chemical stimuli are important in male–female encounters during reproduction[4]. Colony observations in aquaria have shown that after the disruption of an established social situation, there is considerable behavioural interaction until the new social order is stabilised. Subsequent behavioural interaction is much less frequent, although a rank order is maintained. Since most social encounters seem to depend on visual cues, it seemed likely that vision was involved in the subtle interactions which characterise behaviour in an established social order I have therefore monitored and analysed the horizontal eye movements of male *H. burtoni* during social encounters. I found a new kind of very fast body movement, during which the eyes were held fixed, which are correlated with specific types of behavioural interactions.

Mature male *H. burtoni* (7.0–7 5 cm long) were fitted with eye stalks by an adaptation of Easter's technique[5]. Eye stalks were made from black shrink tubing (0.5 mm diameter × 4.0 mm) by squeezing one end closed with heated tweezers Chewing gently on the stalks for a few minutes softened them so that they could be attached by suction to the upper or rear edge of the fish's cornea. The obstruction to the visual field was 10% or less The fish were placed in a cylindrical Plexiglas tank (50 cm diameter × 15 cm) with a transparent floor. The outer circumference was covered with opaque material, and the tank was illuminated from above through a translucent cover. Normally, three to five fish with eyestalks were filmed from below as reflected in a front-surfaced mirror, with a Super-8 Beaulieu camera at 60 frames s^{-1}. An electronic counter driven by a pulse generator at a known frequency was filmed simultaneously for identification of individual frames and calibration of filming speed.

The data were analysed by projecting the film frame by frame and viewing it through a clear Plexiglas disk with parallel lines etched on it and mounted on a potentiometer. The lines were aligned with the object of interest—an eye stalk or the body position—by rotating the disk. The body position was taken as an axis perpendicular to a line drawn between the two eyes. A voltage proportional to the angle being read was punched on paper tape, which was later fed into a computer (DEC 11/40) and the angular orientation plotted against frame (time). The orientation of the body and of each eye of the fish could thus be measured with respect to the tank for each frame. This angular measurement was repeatable with an accuracy of ±1°.

Three types of motion could be distinguished: eye movements without associated body movements, coordinated eye and body movements (both of which have been described before), and fast body movements with no corresponding eye movements. Figure 1*a* illustrates the first type in which the body remains stationary while the eyes flick rapidly to new positions and, after some time (in this case about 0.5 s), return to approximately their original positions. A typical example of the coordinated eye–body movement is shown in Fig. 1*b* A binocular saccade normally preceded a body turn, and the eyes rotated in the opposite direction relative to the body as turning continued, so that they remained in a nearly constant orientation in space. Both types of eye movement have been described for goldfish[5,6].

Fig. 1 *a*, Absolute angular position of the left eye (LE), body (BO), and right eye (RE) as a function of time. The body remains stationary while the eyes rotate to a new position, returning after 0.5 s. *b*, Coordinated eye–body movement during a slow body turn. In this saccadic eye movement, the eye flicks to new fixation points in space which are maintained as the body turns.



The details of such eye movements in *Haplochromis* and experimental analysis of their control and use will be described elsewhere.

Two examples of the third type of motion are shown in Fig. 2. During these fast body turns, the eyes remain fixed relative to the body. In the first case there are two successive fast turns, both about 40°, separated by 0.2 s. Approximately 0.25 s after the second turn, the animal made a typical saccadic eye movement and corresponding slow body rotation. In the second example, the fish made one large fast turn (105°), followed in 0.1 s by a smaller turn (20°). Approximately 1 s later, the fish made a binocular saccade coordinated with a slow body turn. After the fast turn, if slow body movements occurred, the eyes seemed to be fixated in space (compare eye position in Fig. 2*a* between the two turns).

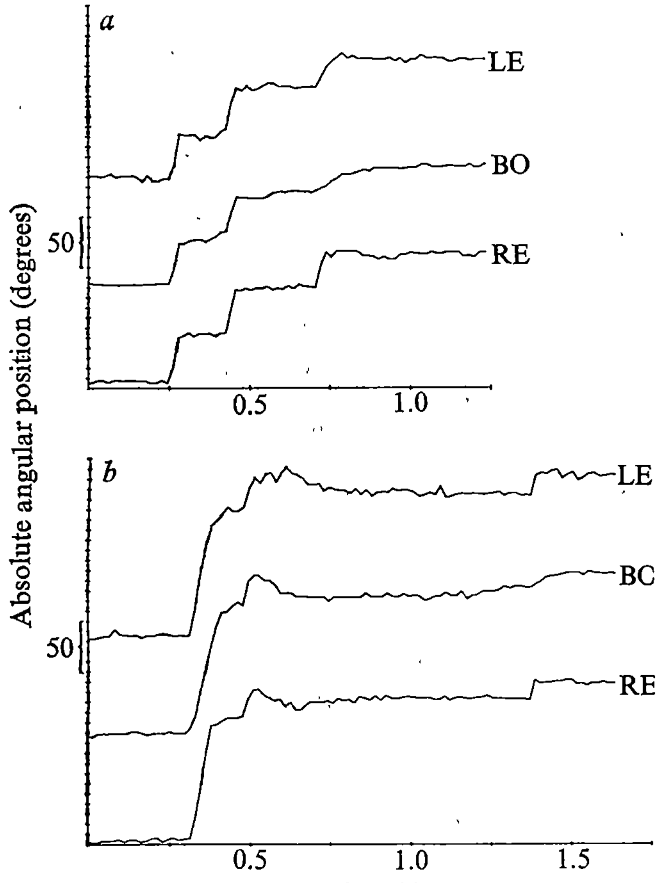


Fig. 2 *a*, Two successive fast body turns followed by a saccadic eye movement (at 0.75 s). During the fast turns there is no eye motion relative to the body. *b*, One large fast turn, followed by a small one and then later (at 1.3 s) a saccadic eye movement.

Both the angular size and angular speed of fast body movements far exceed those of body movements associated with saccadic eye motion (Fig. 3). In fact, the rotational velocity of the body during a fast turn approximates that of the eye during a typical saccadic flick (compare Fig. 2).

Compared with a normal aquarium, the tank I used had a relatively small volume for habitation by several fish, and this artificial crowding intensified the behavioural interaction in response to alteration of the social situation After a fish had been introduced into the tank, there was a short quiescent period of adaptation to the new environment of about 10 min. Some time thereafter, vigorous fighting ensued (threat displays, biting, chasing, mouth-to-mouth biting and pulling), which seemed similar to territorial disputes in larger aquaria. The fighting continued (0.5–1.5 h) until one fish clearly dominated the tank, having won in paired encounters with all contenders. Only rarely was this social order then changed without the addition or removal of fish.

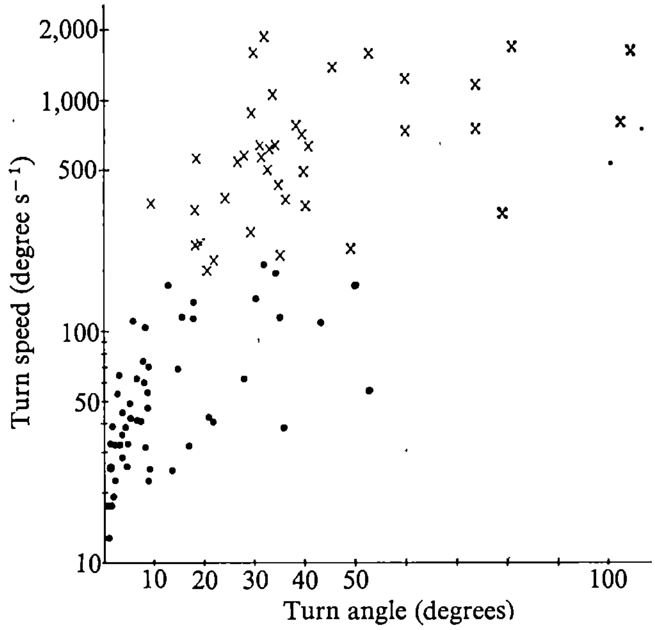


Fig. 3 For body movements, the turn size (degree) is plotted against the turn velocity (degree s^{-1}). Body movements for both fast turn (×, $n = 39$) and saccadic eye movements (●, $n = 52$) are shown.

The fast turns were recorded only during these paired encounters. The animals oriented themselves for subsequent bites, threats and so on with a fast turn just before the behavioural act. The two fast turns in Fig. 2*a*, for example, preceded a vigorous bite into the opponent's tail, after which the attacked animal fled. If the dominant animal was removed from the tank, the remaining fish resumed fighting and performing the corresponding fast turns until a new male dominated.

It is not yet clear whether the fast turn serves as a behavioural signal, intentional or otherwise, in addition to its orienting function. These turns produce a unique sound (Fig. 1 of ref. 3) and can be recognised by a trained observer. The combined auditory and visual information could serve to alert fish nearby.

The fast turns are neither identically long nor large, but seem to be matched to the behavioural situation, which suggests that there is a large coordinated muscular discharge under rather precise control. Two factors make control of this turn unique: first, during the turn, the eyes are fixed with respect to the body, and second, the time spent turning is very short. Both imply that the fish may be making the turns under open loop control, without use of visual or other feedback, a possibility which must be tested experimentally.

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Multicomponent alarm pheromones of the weaver ant

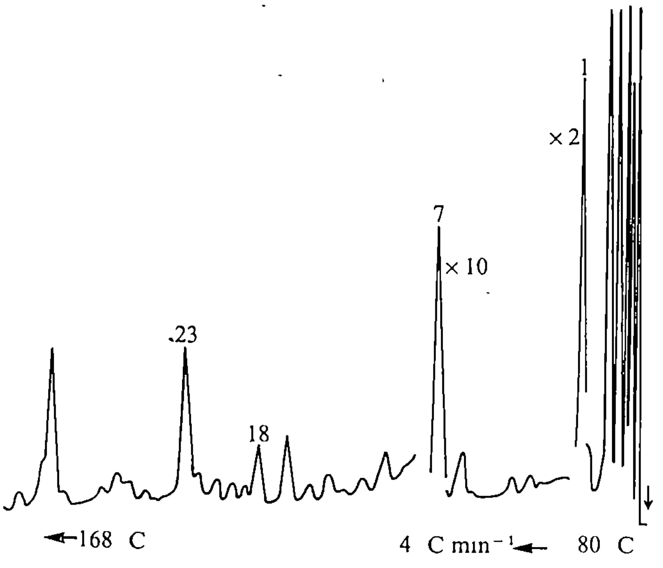
THE exocrine glands of ants are often found to contain blends of volatile chemicals[1], but only rarely have distinct releaser properties been ascribed to different components of a single gland[2–4]. The African weaver ant *Oecophylla longinoda* is notoriously aggressive in defending its territory, and communication of alarm is rapid and efficient. Colony defence is carried out almost exclusively by the major workers, the minor caste remaining within the leaf-nest until this is directly disturbed[5]. This marked difference in behaviour may reflect a biochemical polymorphism, although this has not yet been reported for alarm pheromones of ants[6]. We have therefore initiated a detailed study of exocrine secretions that could be implicated in the control of alarm and defence in this ant. In the mandibular gland we have found over thirty compounds, some of which control separate aspects of behaviour. The mandibular gland contents also differ markedly among castes.

Excited major workers often give out a fragrant but musty odour, which originates from the mandibular glands. Crushed heads or mandibular gland reservoirs release a sequence of behaviour, which may be broken down into four components: alerting, in which the ants raise their heads and open their jaws, and may snap at nest-mates nearby, attraction towards the source of the chemicals, arresting of locomotion at close range, and biting. The last is a particularly marked reaction, in which the ant often pulls for several minutes if resistance is offered by the object being bitten. Prey is normally immobilised by a number of ants pulling from different directions. Gas chromatography of single mandibular glands indicated that at least 33 chemicals are present at or above a level of 5 ng per gland (Fig. 1). The major components were identified as hexanal (1) and 1-hexanol (7). Pure samples of these were presented to laboratory cultures of the ants in capillary tubes, which give a release rate comparable with that produced by the ants[7]. Hexanal releases alerting and alarm with little evidence of directional orientation, while a source of 1-hexanol attracts ants towards it from a radius of about 10 cm, but the ants are alarmed and repelled at a distance of a few mm from the source. The biting behaviour is apparently under the control of less volatile components, since this can be observed in response to the mandibular gland secretion after it has been allowed to evaporate for a few minutes, and no longer releases alarm.

Small quantities of the components eluting after 1-hexanol were collected as broad fractions by solid-sample preparative gas chromatography[8] and were presented to foraging major workers. The fraction containing peaks 18–23 was most active in releasing biting (Wilcoxon matched-pairs signed-ranks test, $T=1$, $N=8$, $P<0.01$, one-tailed test) compared with controls. This fraction was further divided into individual components, and peaks 18 ($T=10$, $N=10$, $P<0.05$) and 23 ($T=0$, $N=7$, $P<0.01$) were most active. They were shown to be 3-undecanone (18) and 2-butyl-2-octenal (23) by mass spectrometry and comparison with commercial (18) and synthetic (23) samples (full details of the structure elucidation will be published elsewhere). The combination of 1-hexanol, hexanal and 2-butyl-2-octenal has also been found in the coreid bug *Amblypelta nitida*[9]; a linked biosynthesis seems likely in both species. 2-Butyl-2-octenal and 3-undecanone were tested in the quantities found in a single gland (50 ng and 20 ng respectively). Both released biting, although the aldehyde was more active than the ketone ($T=14$, $N=12$, $P<0.05$, two-tailed test). The ketone is also an attractant over a 2-cm range.

The behaviour released by the total mandibular gland secretion has been compared with that described for the four chemical stimuli we have isolated (Fig. 2). It seems that a sequential message is produced from the active spaces[10] of these chemicals determined both by their individual volatilities within the mixture and their threshold concentration for the particular response. In still air the spaces will be concentric about the point of deposition of the secretion detailed in Fig. 2. Behavioural tests indicate that hexanal has the most rapidly expanding activity; this is followed by 1-hexanol, which attracts the ants towards the smallest active

Fig. 1 Gas chromatography of the head of a single major worker of *Oecophylla longinoda*; 1.5 m by 6 mm outside diameter glass column packed with 10% polypropylene glycol adipate, the oven held at 80 °C for 5 min, then programmed to 168 °C at 4 °C min^{-1}, injection (arrowed) by solid-sample[11]. Only those components discussed in the text are numbered.



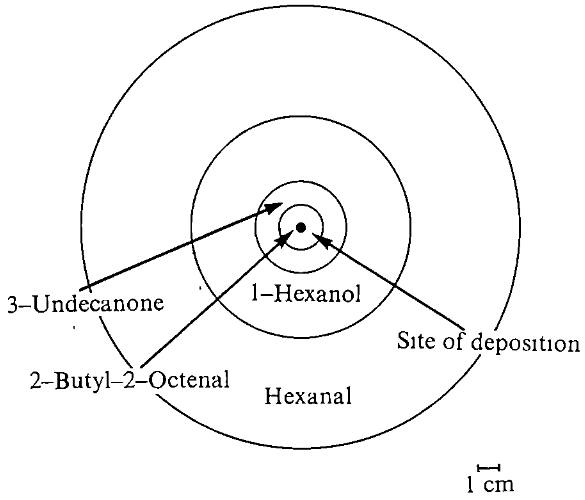


Fig. 2 Active spaces of four mandibular gland components 20 s after deposition. (1) Hexanal and 1-hexanol were presented to foraging workers as pure compounds in glass capillary tubes (1 mm inside diameters) open at one end, which give a release rate comparable with that produced by the ants[7]. After 20 s, the alerting and alarming activity of hexanal extended to a radius of 10 cm, and orientation and running towards a source of 1-hexanol began at a radius of 5 cm. (2) 3-Undecanone and 2-butyl-2-octenal were presented on small flags of filter paper which were first washed in redistilled methylene chloride and were fixed to the foraging table on fine entomological pins. Test solutions of 20 ng of 3-undecanone and 50 ng of 2-butyl-2-octenal (the quantities found in a single mandibular gland of a major worker from the colony under test) were measured on to the filter paper and the solvent (hexane) allowed to evaporate. Control filter papers were treated with solvent only. Over a 20-s period, ants were attracted to 3-undecanone over a 2-cm radius, but biting behaviour in response to either 3-undecanone or 2-butyl-2-octenal was observed only within 1 cm of the pin. Observations on behaviour in response to the total mandibular gland secretion showed that the active spaces of the components were approximately the same in combination as they were when tested individually.

spaces, those of the biting markers. 3-Undecanone may provide an additional directional cue at closer range. The effects of the individual components seem to be additive, rather than synergistic, as observed in two laboratory cultures, collected from the forest areas of Ile-Ife and Ibadan, Nigeria. Pure 3-undecanone released biting in a third culture, collected about 700 km to the north, in savanna at Mokwa, but 2-butyl-2-octenal was inactive alone. A sample containing an equal amount of the aldehyde and 1-hexanol restored the activity to the same level as the ketone: mandibular glands of major workers collected from Mokwa differ in containing 1-octanol and 1-nonanol in addition to 1-hexanol, and these less volatile alcohols may be more persistent synergists. In either case a rapid communication of alarm is achieved, while the attack response is localised to the object on to which the mandibular gland secretion has been deposited.

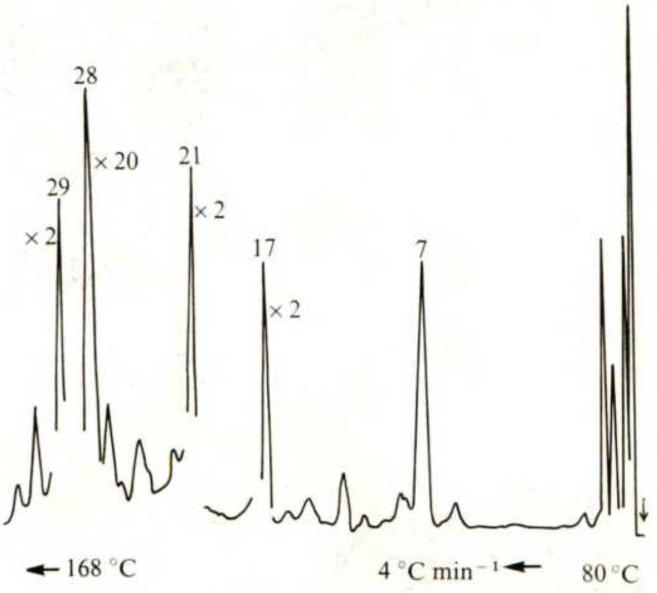


Fig. 3 Gas chromatography of the head of a single minor worker head of *Oecophylla longinoda;* experimental details as for Fig.1, vertical scale increased × 2.

Gas chromatography of single heads of minor workers (Fig.3) indicates considerable differences from majors; the main component (28) was identified as nerol, with geraniol (29), 1-hexanol (7), 1-octanol (17) and 1-nonanol (21) also present. Hexanal, 3-undecanone and 2-butyl-2-octenal seem to be absent at the level detected (~2 ng per ant). Minor workers exposed to either 1-hexanol and nerol in capillary tubes are repelled, and thus displaced from sources of disturbance. Major workers are attracted and arrested at about 1 cm from a source of nerol in a capillary, but this reaction is not observed until about 2 min after the tube is placed on the foraging table, which indicates a high threshold concentration. A disturbance within the confined space of the leaf-nest would result in a rapid and rather uniform increase in the concentration of 1-hexanol, decreasing its effectiveness as an orientation stimulus. For the same release rate, the active space of nerol is smaller and slower to expand, and this could enable excited minor workers to be located by majors.

Two further methods of alarm communication are found in this species. Major workers strike their bodies against the leaf-nest when it is disturbed, the sound being likened by Weber[5] to dry peas dropping on to a plate, and several major workers then appear at the nest entrance. The abdominal defensive secretion also releases alarm behaviour, but only two compounds seem to be involved. A mixture of formic acid, found in the poison gland, and *n*-undecane from the Dufour gland, releases alarm, attraction and attack. The mixture is considerably more effective than either component tested separately. The poison and Dufour gland secretions of major and minor workers are qualitatively similar.

This study has focused on only a few of the many chemicals which may have a role in the control of alarm and defence behaviour in this species, and suggests that the simple concept of an alarm pheromone governing behaviour by virtue of one major component may be inappropriate for ants. It also demonstrates that biochemical polymorphism and population differences exist and should be taken into account in future studies.

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Penetration of mid-gut cells of *Glossina morsitans morsitans* by *Trypanosoma brucei rhodesiense*

UNTIL recently, the developmental cycle of African sleeping sickness trypanosomes in tsetse flies (*Glossina* spp.) was thought to follow exclusively the pattern described by Robertson[1]. She suggested that trypanosomes ingested by a fly taking an infected blood meal multiplied in the gut and eventually invaded the salivary glands directly without leaving the confines of the alimentary tract. Inside the salivary glands the trypanosomes could develop into metacyclic forms capable of initiating fresh infections in mammals. In 1972, Mshelbwala[2] described stages of *Trypanosoma brucei* he had found in the haemolymph of three species of tsetse fly, and suggested that a more direct route from the gut to the salivary glands existed. According to his hypothesis, the trypanosomes from the gut of the fly penetrated the gut wall and entered the haemocoel; they then migrated to the salivary glands directly through the haemocoel, penetrating the glands and so completing the cycle of development in the fly. At this time there was no direct evidence that salivarian trypanosomes could penetrate the gut wall of their insect vector. In this communication we present electron microscopic evidence that *T. b. rhodesiense* penetrates the mid-gut cells of *G. morsitans morsitans*.

Tsetse flies are difficult to infect with trypanosomes of the *T. brucei* complex, and one suggested[3] reason for this is failure by the trypanosomes to complete the biochemical and morphological changes by which the bloodstream trypanosomes transform to mid-gut forms. To overcome this difficulty, we cultured *T. b. rhodesiense in vitro*, producing forms which are believed to correspond to mid-gut forms, which were then fed to the flies.

Laboratory-fed *G. m. morsitans* were fed artificially through agar–Parafilm membranes[4] on a diet of defibrinated horse blood containing approximately 2×10^5 culture-form trypanosomes per ml. These culture forms were grown to mid-log phase in a human blood lysate broth medium[5], and were derived from a strain of *T. b. rhodesiense* (180 TS1) isolated by Dr W. E. Ormerod from a human patient in Botswana. The flies which provided material for the electron micrographs presented here were fed on six occasions. The first three feeds

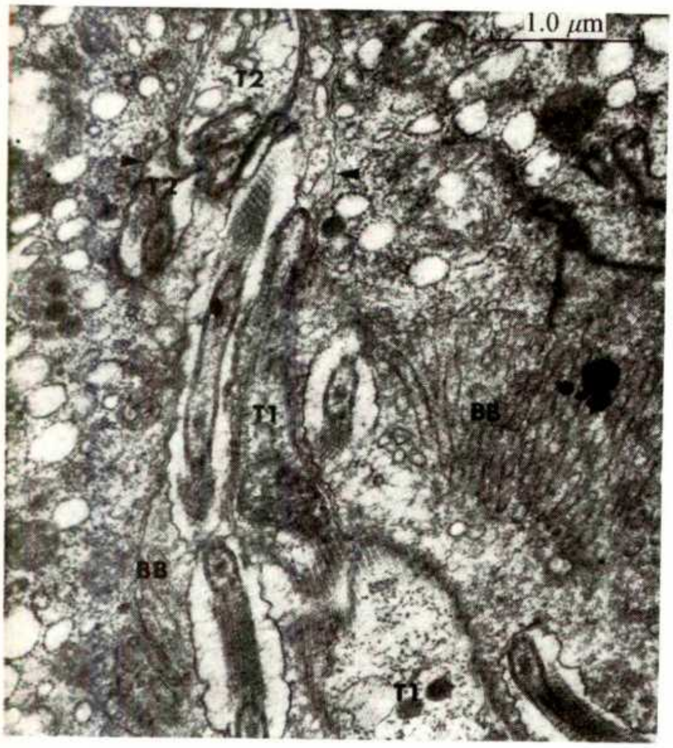


Fig. 1 Two trypanosomes (T1 and T2) invaginating the brush border membrane (BB). The walls of the resulting pocket are arrowed.

Fig. 2 Transverse section of two invaginated pockets in a gut-cell containing numerous trypanosomes. The membranes of these pockets are clearly seen unlined with microvilli.

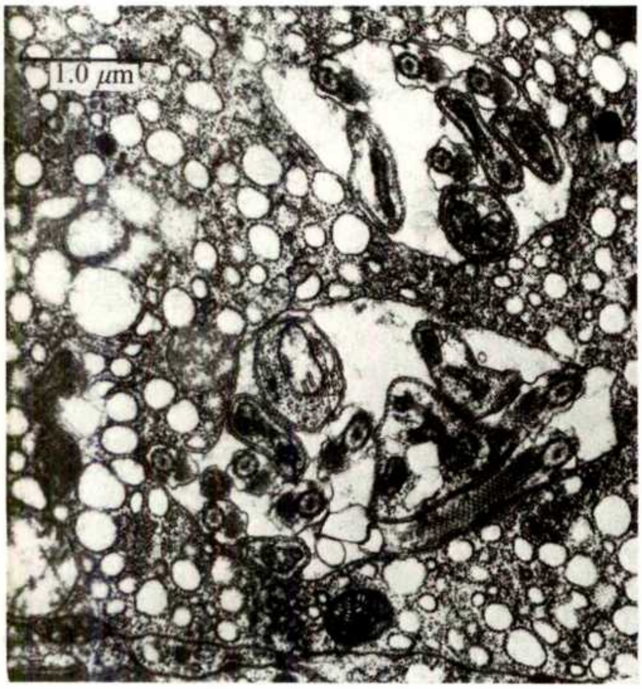


Fig. 4 *a*, Higher magnification of a trypanosome pellicle ▶ (arrowed) showing the parasite within the gut cell and free of any host-cell membrane. *b*, Two trypanosomes lying between the mid-gut basement membrane and the membrane of the haemocoel (arrowed). All sections were stained with lead citrate.

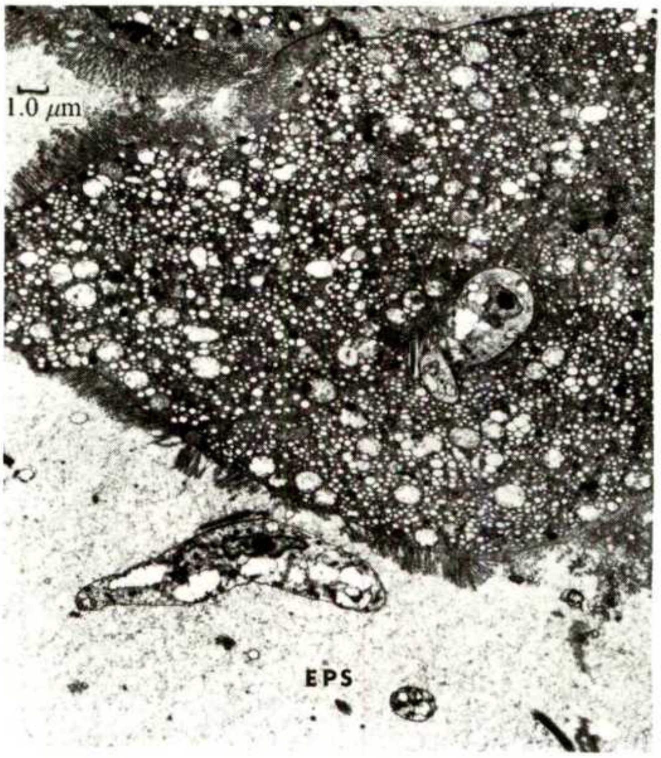
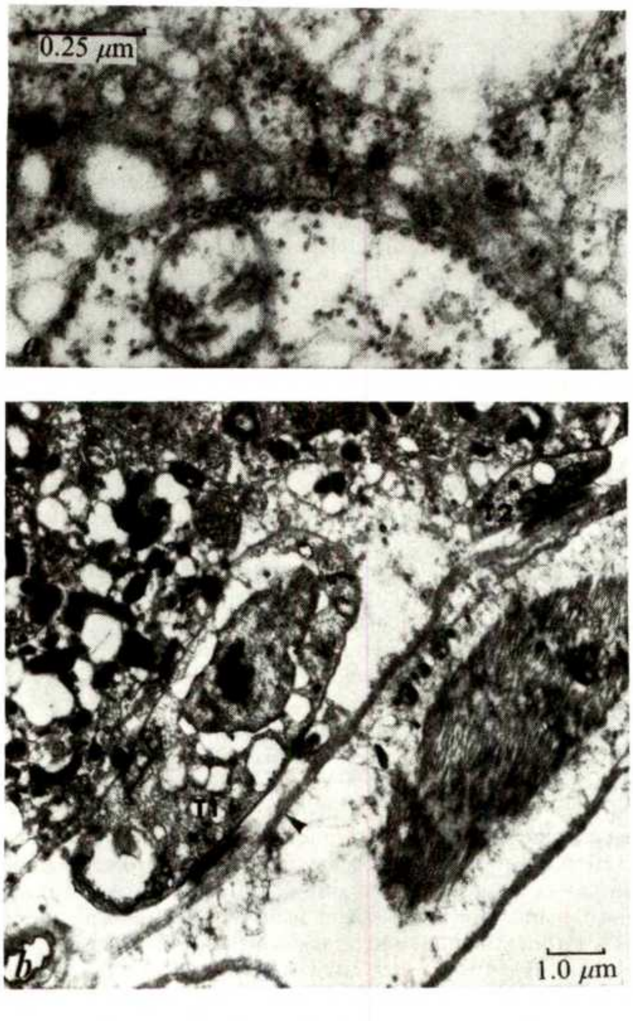


Fig. 3 Two trypanosomes within the cytoplasm of a mid-gut cell. Other trypanosomes are visible in the ecto-peritrophic space (EPS).



were of trypanosome-infected blood, and the remaining feeds contained defibrinated blood alone. Twenty-one days after their first infected feed, the flies were killed and their guts dissected out. The guts were fixed in 3% (v/v) cacodylate-buffered glutaraldehyde (6 h at 4 °C), washed overnight in cacodylate buffer, postfixed in cacodylate-buffered 1% (w/v) osmium tetroxide and prepared for embedding in Araldite.

The electron micrographs presented here show trypanosomes in the ecto-peritrophic space of the mid-gut, close to the brush border of the gut epithelium, and penetrating, flagellum first, the brush border membrane. This membrane, now surrounding the trypanosome flagellum (Fig. 1), has the appearance, when viewed in transverse section (Fig. 2), of a vacuole within the host cell containing numerous parasites. Once wholly inside the gut cell (Fig. 3), the trypanosomes lie free, no longer surrounded by host cell membrane (Fig. 4*a*).

Finally, the trypanosomes reach the basement membrane of the gut cell, which they penetrate, and come to lie in the space between the gut cell basement membrane and the haemocoel membrane which sheaths the gut (Fig. 4*b*).

It seems that entry of the trypanosomes into tsetse fly mid-gut cells is an active process on the part of the trypanosomes, and not a result of phagocytic action of the mid-gut cells, nor was evidence found of passive enclosure of trypanosomes between gut cells during the extension and contraction following blood meals. This view is supported by our finding that the intracellular parasites are in no way associated with the membranous system of the host cell; the electron microscopic appearance of the intracellular trypanosomes, and the discovery of undamaged parasites in the space between the gut cell basement membrane and the haemocoel membrane, suggest that the trypanosomes are not affected by the gut cells.

A route from the gut of an insect vector through the haemocoel to the salivary glands has been described for other parasitic protozoa. *T.* (*Herpetosoma*) *rangeli* migrates to the salivary glands of its reduviid bug vector in this manner, as do malaria sporozoites in the mosquito. Whether this route is important for sleeping sickness trypanosomes is unknown, but this is being investigated.

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T cells mediate transplantation tolerance

SEVERAL cellular mechanisms have been postulated to account for the phenomenon of transplantation tolerance in animals injected at birth with allogeneic or semi-allogeneic cells. These include deletion of the clone of specific alloantigen sensitive cells[1], continuous production of conventional blocking alloantibody[2] or anti-receptor antibody[3] and the action of suppressor cells[4]. Although these phenomena have been demonstrated in tolerant animals, it has never been shown that either alone or in combination they are actually capable of inducing true transplantation tolerance. In the course of experiments designed to prove formally that recirculating small lymphocytes are responsible for first-set rejection of skin grafts, we obtained results suggesting that rapidly recirculating cells from tolerant donors might be capable of actively transferring a state of transplantation tolerance (TT) to irradiated recipients[5,6]. These results, which suggested that the transfer of TT was due to humoral or cellular suppressor mechanisms, were not consistent with the clonal deletion theory of tolerance. The present experiments were designed to confirm that TT is not merely the absence of reactivity to a given set of allo-antigenic determinants but an active suppression of reactivity to those determinants, and to determine whether T or B cells mediate this suppression.

Thoracic duct (TD) lymph contains both T and B cells and members of both these subpopulations continuously recirculate from blood to lymph[7]. Cells mediating allo-immune responses *in vivo* are among the populations which recirculate[5,6]. The lymphoid tissues of rats whose own lymphocyte pools have been virtually eliminated by supralethal (1,000 rad) doses of radiation are still capable of supporting the recirculation from blood to lymph of injected TD lymphocytes. Either syngeneic or allogeneic TD lymphocytes injected intravenously into such rats can be recovered from a thoracic duct fistula uncontaminated by significant numbers of radioresistant host lymphocytes[6]. Because the modal transit time of T lymphocytes through lymph nodes is considerably shorter than that of B cells[8,9], peak recovery of injected T cells occurs before significant numbers of B cells begin to emerge (Fig. 1). Collections of lymph made for the first 24 h after injection yield approximately 20% of the injected T cells and virtually none of the injected B cells. We have examined the capacity of both unfractionated TD lymphocytes from tolerant donors, and pure T-cell populations separated from the former by this method, to mediate TT.

Thoracic duct lymph from 10 DA rats tolerant of PVG alloantigens was collected for the first 24 h after cannulation and the cells were washed twice. Aliquots of 3×10^7 cells were injected intravenously into sublethally (750 rad) irradiated DA rats freshly grafted with PVG and Lew skin. The remainder of the cells (1.25×10^9 per recipient) were injected intravenously into supralethally irradiated DA rats bearing thoracic duct fistulae, and collections of lymph

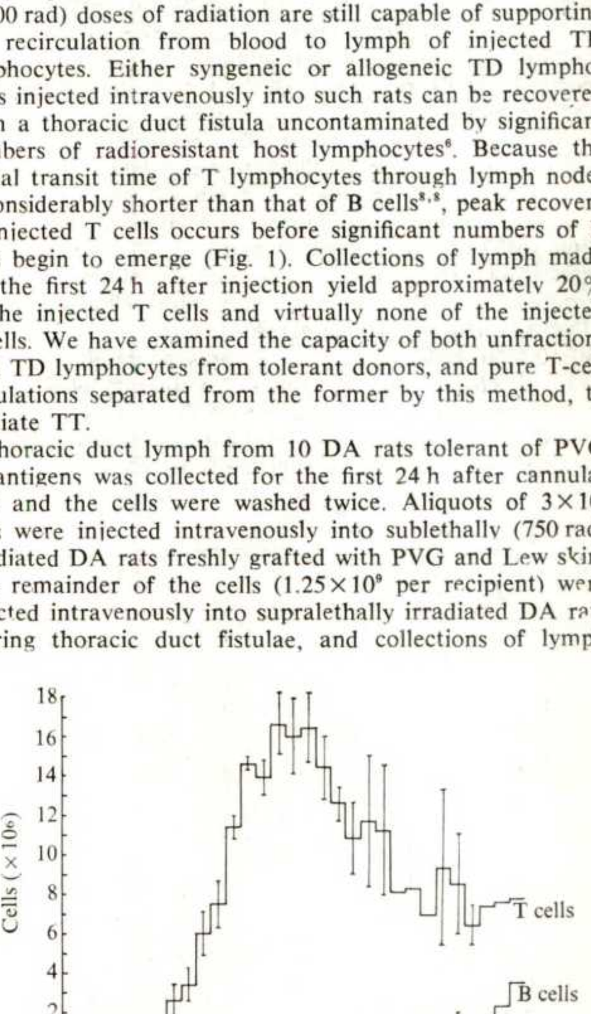

Fig. 1 Output of T and B cells in 1.5-h collections of TD lymph from supralethally irradiated rats (1,000 rad ^{60}Co γ radiation) injected intravenously with 1.25×10^9 syngeneic TD lymphocytes of which 44–48% were Ig positive. All rats were splenectomised at the time of cannulation to prevent temporary sequestration of injected TD lymphocytes in the splenic spool. Cells were counted in each collection and the proportion of Ig-bearing lymphocytes was determined by direct fluorescence using FITC conjugated rabbit antirat Ig serum diluted 1:30 (Burroughs Wellcome, lot No. K7284). T cells were positively identified in representative collections by indirect immunofluorescence using a 1:4 dilution of a rat anti-T-cell serum[15] followed by two washes through foetal calf serum gradients and staining with the same rabbit anti-rat Ig serum as before. Error bars represent the standard errors of the arithmetic means of determinations on eight rats in four separate experiments.

made from these rats for the subsequent 0–22 h and 22–46 h. Cells in each of these collections were pooled and assayed for the proportion of T and B cells. Aliquots of 3×10^7 of these cells were injected intravenously into sublethally irradiated DA rats also bearing both PVG and Lew skin allografts (Fig. 2).

In order to determine the rate of spontaneous recovery of immune responsiveness in rats given 750 rad, groups of irradiated, unrestored rats were doubly grafted in the same way as the experimental groups. In addition, the competence of separated T cells from normal donors to mediate alloimmune responses was confirmed by restoring a further group of irradiated, doubly grafted recipients with 3×10^7 cells from supralethally irradiated DA rats given 1.25×10^9 TD lymphocytes from normal donors. The survival of skin grafts on rats given normal or tolerant T-cell inocula and on rats given no restorative inoculum are presented in Fig. 2. As the behaviour of grafts on rats given unfractionated TD lymphocytes, either normal or tolerant, did not differ from the results obtained with the respective purified T cells, the results of the former are omitted from the life tables for the sake of clarity.

In the unrestored sublethally irradiated control rats, spontaneous recovery of the immune response ensured that both grafts were eventually rejected, although the first-set allograft response was temporarily ablated. Median survival time (MST) for Lew grafts was 17.5 d; the MST for PVG grafts was 25.3 d (Fig. 2, *D* and *E*). When sublethally irradiated recipients were restored with cells from normal donors, whether unfractionated TD lymphocytes containing 40–50% B cells or purified T cells containing less than 0.3% B cells, the rejection times of both grafts were restored to normal (MST Lew, 8.5 d; MST PVG, 9 d) (Fig. 2, *A* and *B*). Previously reported experiments have established that the restored responses in sublethally irradiated grafted rats given TD lymphocytes are mediated by the restorative inoculum[5,6], and that if the clone of cells reactive to PVG antigens is deleted from such a restorative inoculum by filtration through supralethally irradiated PVG rats, the restoration of first-set rejection of PVG skin grafts is specifically abolished; these grafts are rejected with the same MST as in unrestored sublethally irradiated animals[6].

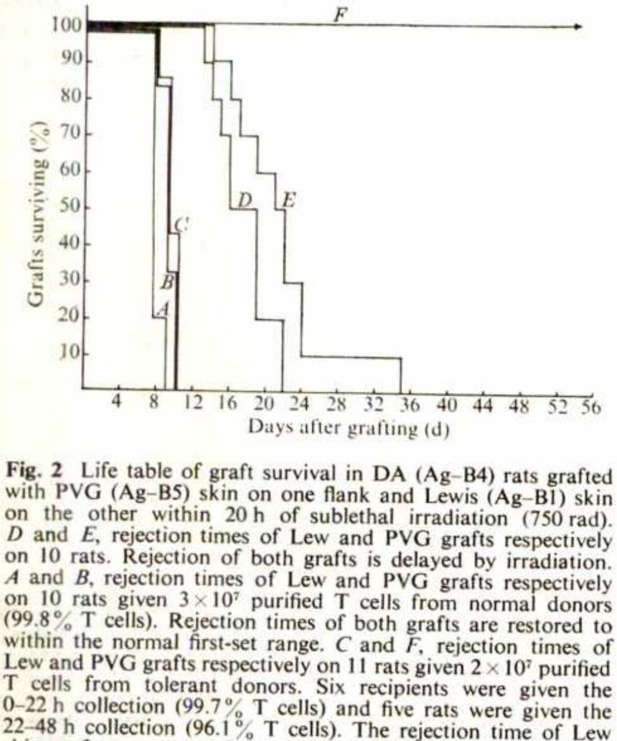

Fig. 2 Life table of graft survival in DA (Ag-B4) rats grafted with PVG (Ag-B5) skin on one flank and Lewis (Ag-B1) skin on the other within 20 h of sublethal irradiation (750 rad). *D* and *E*, rejection times of Lew and PVG grafts respectively on 10 rats. Rejection of both grafts is delayed by irradiation. *A* and *B*, rejection times of Lew and PVG grafts respectively on 10 rats given 3×10^7 purified T cells from normal donors (99.8% T cells). Rejection times of both grafts are restored to within the normal first-set range. *C* and *F*, rejection times of Lew and PVG grafts respectively on 11 rats given 2×10^7 purified T cells from tolerant donors. Six recipients were given the 0–22 h collection (99.7% T cells) and five rats were given the 22–48 h collection (96.1% T cells). The rejection time of Lew skin grafts was restored to within the normal first-set range in both groups. The rejection of PVG skin grafts in both groups was completely suppressed. These grafts are in impeccable condition at the time of writing (> 100 d). Tolerant donors had borne perfect PVG skin grafts for at least 50 d. Pooled TD lymphocytes from these donors were assayed for graft *versus* host activity in $(DA\times PVG)F_1$ and $(DA\times Lew)F_1$ hybrids using the popliteal lymph node assay[16]. Absence of responses in $(DA\times PVG)F_1$ and large responses in $(DA\times Lew)F_1$ confirmed true tolerance in the donors.

The effect of inocula of cells tolerant to PVG antigen in sublethally irradiated recipients was markedly different (Fig. 2). The recovery of reactivity to PVG antigens was not merely delayed, as in unrestored recipients or recipients of clonally deleted cells, but the PVG grafts survived indefinitely in impeccable condition. This phenomenon is not explicable in terms of absence of the clone of alloantigen sensitive cells but implies that populations of tolerant cells contain cells capable of active, specific suppression of the regenerating immune response in animals recovering from radiation.

Both suppressor T cells and specific B-cell products have been implicated as mediators of tolerance in a variety of systems[10]. Tolerant TD lymph contained the same proportion of B cells, 40–50%, as does normal TD lymph. When passaged from blood to lymph in supralethally irradiated rats, 99.7% of the tolerant TD lymphocytes collected within 24 h of injection were T cells. Both this population and later collections (96.1% T cells) were just as efficient as unfractionated, tolerant TD lymphocytes in adoptively transferring specific tolerance (Fig. 2, *C* and *F*). The original donors of tolerant TD lymphocytes had been given 5×10^7 $(DA\times PVG)F_1$ bone marrow cells by intracardiac injection within 24 h of birth and grafted with PVG skin at 12 weeks. The extent of chimaerism in these donors (assessed by dye-exclusion cytotoxicity assays using specific cytotoxic alloantisera of high titre) was below the detection threshold of 5%. This is in agreement with the low levels of chimaerism in tolerant mice reported by other workers using different assay systems[11,12]. Experiments were nevertheless done to exclude the possibility that chimaeric cells in the restorative inocula from tolerant donors could have been responsible for the induction of tolerance in irradiated hosts. The rejection of PVG skin grafts by sublethally irradiated rats was completely unaffected by giving restorative inocula of either 3×10^5 or 3×10^6 $(DA\times PVG)F_1$ cells. The latter would be equivalent to 10% chimaerism. This makes it unlikely that the transfer of chimaeric cells is responsible for the suppressor activity of purified T-cell populations from tolerant donors.

A dose of 750 rad is far in excess of that necessary to abolish completely alloantibody synthesis in rats[13], and restorative inocula containing 20–40 times as many B cells as were present in the restorative inocula used in these experiments will not restore antibody synthesis to such animals[14]. It therefore seems clear that the injected syngeneic T cells alone must have been responsible for the specific suppression which induced tolerance in the irradiated recipients of tolerant T-cell inocula. This result implies that true transplantation tolerance is a positive immune response mediated by T cells which belong to the recirculating pool of small lymphocytes. The origins, life span and mode of action of these cells will obviously be the subjects of further study.

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Clonal growth of Hodgkin cells

ALTHOUGH several cell types derived from biopsies of patients with Hodgkin's disease have been grown *in vitro*[1,2], there has been no convincing experimental evidence for the identity and origin of such cells. We wish to present data demonstrating clonal and continuous proliferation of Hodgkin cells, their relationship to Reed–Sternberg cells and their probable origin from B lymphocytes.

Cells were obtained from pleural effusions of two patients with terminal Hodgkin's disease. The lymph node histology at autopsy of both cases was lymphocytic depletion. The cases were selected on the basis of an abundance of Hodgkin and Reed–Sternberg cells in the pleural fluid (Fig. 1): the large Reed–Sternberg cells were binucleate or multinucleate, the nuclei having a coarse nuclear chromatin network and large blue-stained nucleoli. Their cytoplasm was pale and often vacuolated. Cells considered to be Hodgkin cells had the same nuclear and cytoplasmic characteristics, but were mononuclear.

Cells were separated from the pleural effusions by the Ficoll gradient method and cultured in diffusion chambers as described for human bone marrow and peripheral blood cells[3,4]. Figure 2 shows similar growth curves of the cells from both cases during 11 d of culture. An initial decrease in total cell numbers, levelling off on days 2–5, was followed by a steady exponential increase. Net gain of total cells was observed after 8 d in culture in case 2 but not in case 1. During exponential growth, cell doubling times were 48 and 72 h respectively in the two cultures.

After 11 d of culture the cell contents of single diffusion chambers from case 2 were transferred into five to eight new chambers. Subculture was repeated at 11–15 d intervals. This cultured cell line has now been maintained for 4 months with continuous production of cells. Throughout the culture period the proliferative properties of the total population, the morphology of the Hodgkin and Reed–Sternberg cells (Fig. 1) and their proportional distribution (∼30 : 1) remained unchanged.

In both cases the EBNA-test[5] for detection of the Epstein–Barr virus (EBV) genome was negative on the fresh pleural effusion cells.

For chromosome analyses of fresh pleural cells and of cells cultured in diffusion chambers, the direct method was used as before[6]. Contents of three to five diffusion chambers were pooled. G banding was obtained by the trypsin–saline–Giemsa technique[7]. The number of metaphases analysed and the numerical chromosomal data are shown in Table 1. The karyotype of the predominant mode of case 1 was 46,XY,t(3,13),−11,+mar,+mar. One marker chromosome was submetacentric, A_1-sized and had two central bands on the long arms. The other marker was subacrocentric, B-sized and had two proximal bands and one distal band. On day 11 of culture, 8% of the metaphases had 47 chromosomes, the extra chromosome being a No. 21. Otherwise the chromosomal findings of case 1 on days 0 and 11 were very similar, particularly with regard to the translocation and the marker chromosomes which were found in 100% of metaphases. Polyploid metaphases revealed two or three sets of the marker chromosomes. Identity of the translocation chromosome and the marker chromosomes in both preparations was confirmed by their G-banding patterns. The chromosome preparation from a lymph node, removed immediately after death, yielded only one analysable meta-

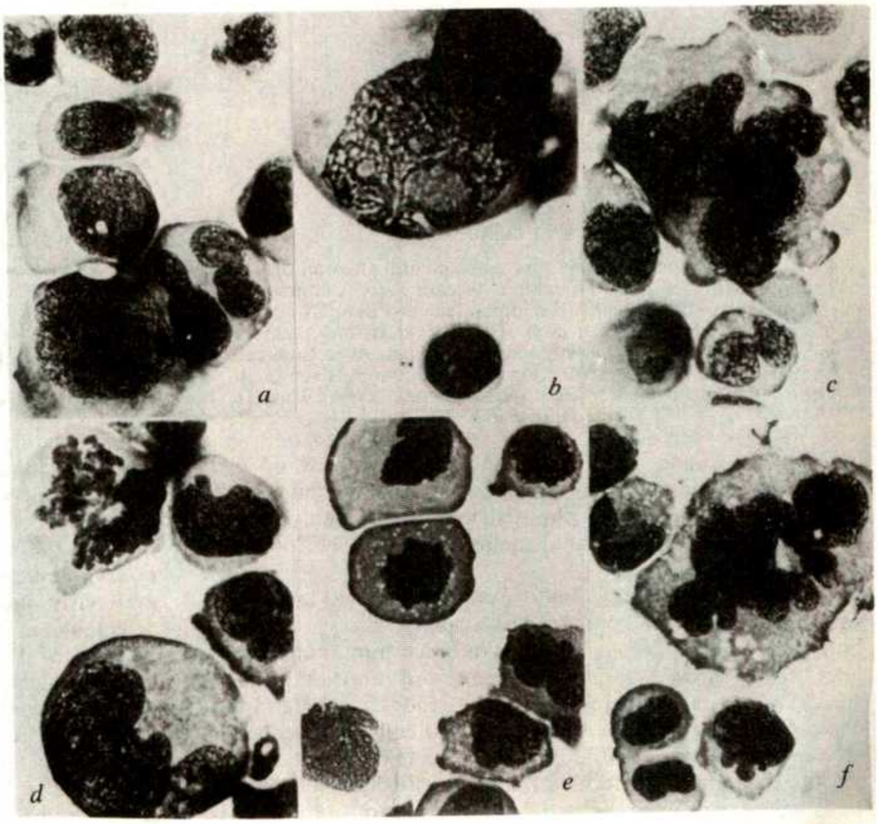

Fig. 1 Composite micrograph showing Hodgkin (HC) and Reed–Sternberg cells (RSC) of case 2. HC and RSC from the original pleural effusion (*a–c*) and after 112 days in diffusion chamber culture (*d–f*). Note normal lymphocyte in comparison to HC (*b*) and mitotic figures of HC (*d–f*). Original magnification on 24 × 36 mm negative film × 400 (*a*, *c*, *d–f*) and × 630 (*b*). May–Grünwald–Giemsa stain.

Table 1 Summary of numerical chromosomal findings of cases 1 and 2

	Day 0		Day 11		Day 50	
	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2
No. of metaphases counted	255	100	50	50	0	100
No. of chromosomes per metaphase						
46	86.0%	0	84.0%	0	—	0
47	0	0	8.0%	0	—	0
55–77	4.4%	93.0%	0	100.0%	—	95.0%
82–92	6.6%	0	8.0%	0	—	0
110–130	2.2%	6.0%	0	0	—	4.0%
174–186	0.8%	1.0%	0	0	—	1.0%

phase which, however, clearly demonstrated the translocation and the marker chromosomes described above.

In case 2, G banding revealed up to 17 marker chromosomes in the near triploid metaphases, the origin of most of which remained obscure. The most prominent and consistent among the marker chromosomes were a large submetacentric and two slightly different subacrocentric chromosomes. Some metaphases showed a 1q- and a 6q-. All metaphases had supernumerary chromosomes 19 and one or more missing 13. Karyotypic characteristics of the near triploid metaphases were invariably demonstrable in duplicate or triplicate in the pentaploid or octoploid metaphases. With regard to distribution of chromosome numbers, karyotypic characteristics and banding patterns, the results of chromosome analyses on days 11 and 50 in culture were in agreement with those obtained on day 0.

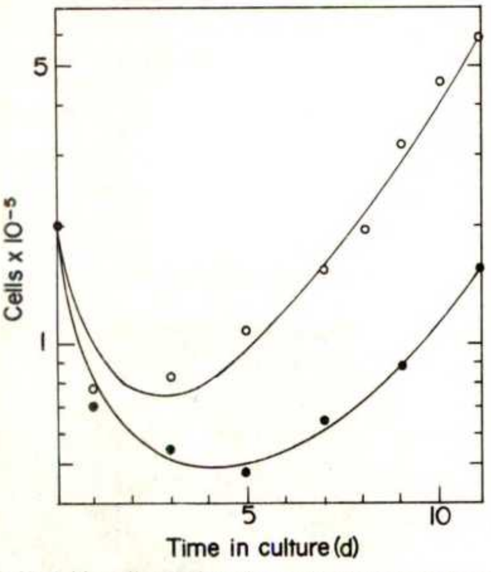

Fig. 2 Hodgkin cells obtained from the pleural effusion of two patients with terminal Hodgkin's disease were cultured in diffusion chambers which were implanted into the peritoneum of irradiated NMRI mice (800 r. ^{60}Co 1 d before chamber implantation), and reimplanted on day 8. Changes in total cell number are shown as a function of time. Symbols represent mean cell number of six to eight diffusion chambers. ●, Case 1; ○, case 2.

On days 0 and 50 the chromosome numbers of 400 further metaphases were estimated at low magnification. The results substantiated the distribution of ploidy values given in Table 1. No normal metaphase could be found at any time in either case.

Cell surface immunoglobulins were determined as before, using an antiserum prepared in rabbits against human immunoglobulins G, A and M[8]. Indirect immunofluorescence tests showed a bright membrane fluorescence in 86% (case 1) and 79% (case 2) of the Hodgkin cells and the Reed–Sternberg cells. We looked at 300 cells of case 1 and 200 cells of case 2. This fluorescence was demonstrated both on the cells directly obtained from the effusions and on the cells collected from the chambers after 50 d of culture. It is thus less likely that immunoglobulins bound secondarily to the cell surface were detected. Spontaneous rosette formation with sheep red blood cells was studied by standard techniques[9]. Neither the Hodgkin nor the Reed–Sternberg cells were positive.

Our findings show that the Hodgkin cells we investigated are capable of continuous monoclonal growth. When cultured in diffusion chambers their growth is exponential, with doubling times of 48–72 h. Chromosome analyses suggest that diploid and triploid Hodgkin cells are precursor cells of the polyploid Reed–Sternberg cells. The demonstration of immunoglobulins on the cell surface even after weeks in culture and the absence of rosette formation point to a B-cell origin of both Hodgkin and Reed–Sternberg cells.

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Absence of infectious Epstein–Barr virus in blood in acute infectious mononucleosis

CULTURES of peripheral leukocytes from patients with infectious mononucleosis (IM) readily give rise to B lymphoblastoid cell lines containing Epstein–Barr virus genome. Recent experiments have shown that such lines arise not through the direct outgrowth of cells infected by EB virus *in vivo* but as a result of the transformation *in vitro* of previously uninfected B lymphocytes by infectious virus detectable in the cultures at an early stage[1,2]. For in cocultures of IM leukocytes and an equal number of foetal leukocytes, the cell lines which emerged were exclusively of foetal origin in 6 out of 16 cases and of mixed origin with foetal cells frequently predominating in the remaining 10 cases; yet foetal cells cultured alone never gave rise to lines[1]. More important, the inclusion of neutralising antiserum to EB virus in the medium inhibited the establishment of cell lines both in cocultures of IM and foetal cells and in cultures of IM cells alone[1,2].

The source of the infectious virus was not established absolutely, and one obvious possibility was that it had come directly from the blood of the acute IM patients where infectious particles could already have been circulating either free in the plasma or sequestered in leukocytes. The present experiments argue against this and support the idea that infectious virus originates from a small number of peripheral IM lymphocytes which carry the EB viral genome in a non-infectious form *in vivo* but which are activated to a cycle of virus production *in vitro*.

Heparinised blood samples (30 to 60 ml) were taken from seven patients with acute heterophile antibody-positive IM between 1 and 4 weeks after the onset of clinical symptoms; serum samples were taken at the same time to investigate the EB viral antibody status of each patient as regards neutralising activity[1], anti-viral capsid antigen (VCA) titre[3], and anti-nuclear antigen (EBNA) titre[4].

About 5 ml of each blood sample was immediately centrifuged at 1,000*g* for 10 min and the supernatant plasma was rapidly frozen, and stored at −70 °C. The remaining blood was centrifuged on Ficoll–isopaque, and the mononuclear cells were collected and washed as previously described[1], and then divided into three samples for the following treatments: (1) Some washed mononuclear cells were suspended in medium (Eagle's minimum essential with 15% foetal calf serum), cultured in plastic vials at a concentration of 2×10^6 to 3×10^6 cells ml^{-1} and observed regularly for 8 weeks for evidence of transformation, as in earlier work[1]. (2) Half the remaining washed mononuclear cells were used to prepare an extract (day 0 extract); the cells were suspended in the medium at a concentration of 1.5×10^8 to 2×10^8 ml^{-1}, disrupted by three cycles of rapid freezing and thawing, and centrifuged at 5,000*g* for 20 min to deposit the debris, after which the supernatant was collected and stored at −70 °C. (3) The rest of the washed cells were cultured for 3 d and were then similarly extracted (day 3 extract) in an equal volume of medium to that used on day 0.

Table 1 Incidence of transformation in cultures of foetal leukocytes exposed to plasma and to extracts of mononuclear cells from acute IM patients

Patient no.	Plasma preincubated with EB virus negative serum	Plasma preincubated with Antiserum to EB virus	Day 0 extract preincubated with EB virus negative serum	Day 0 extract preincubated with Antiserum to EB virus	Day 3 extract preincubated with EB virus negative serum	Day 3 extract preincubated with Antiserum to EB virus
IM71	0/8	0/8	0/8	0/8	1/8	0/8
IM72	0/8	0/8	0/8	0/8	2/8	0/8
IM74	0/8	0/8	0/8	0/8	6/8	0/8
IM76	0/8	0/8	0/8	0/8	0/8	0/8
IM92	0/8	0/8	0/8	0/8	2/8	0/8
IM94	0/8	0/8	0/8	0/8	0/8	0/8
IM96	0/8	0/8	0/8	0/8	3/8	0/8

The plasma and the two cell extracts from each patient were assessed for their ability to transform foetal leukocytes in culture. Samples of each preparation were incubated for 60 min at 37 °C with either antiserum to EB virus of known neutralising activity or negative serum; the mixtures were then shaken with fresh foetal cord blood leukocytes for 60 min after which the foetal cells were washed, set up in microtestplate cultures, and observed for 8 weeks for evidence of transformation. The techniques used have already been described[1].

The incidence of transformation of the foetal leukocytes after exposure to the various preparations is shown in Table 1. No transformation occurred in any culture of foetal cells exposed to IM plasma or extracts of fresh IM mononuclear cells (day 0 extracts). In contrast, five out of seven IM mononuclear cell extracts prepared after 3 d of culture (day 3 extracts) showed detectable leukocyte-transforming ability; this ability obviously depended on the presence of infectious EB virus since it was only manifest with those extracts pretreated with negative serum and was abolished by pretreatment with neutralising antiserum to the virus. Confirmation of the role of EB virus in the transformation was provided by the finding of EBNA[5] expression in the transformed cells.

Table 2 shows the incidence of, and mean time to, transformation in the cultures of washed mononuclear cells from each patient. It is of interest that those mononuclear cell populations which showed the highest and earliest incidence of transformation when cultured on their own (Table 2) were just those which produced infectious EB virus in culture by the time they were extracted on day 3 (Table 1); conversely the two mononuclear cell samples whose day 3 extracts lacked detectable transforming ability also showed the lowest incidence of transformation when cultured alone.

This correlation confirms and extends similar observations in earlier experiments[1]. Table 2 also shows that by the time the patients were investigated in the present study all seven had developed low, but detectable, neutralising antibodies to EB virus, seven had antibodies to VCA, and two had low anti-EBNA titres, but there is no obvious relationship between the serological status of the patients and the behaviour of their mononuclear cells in culture. Nevertheless, the range of serological findings among these seven IM patients is in agreement with results from larger studies[4,6].

Thus, the present experiments show that IM patients with clinically manifest disease do not have infectious EB virus in the circulation. At this stage infectious virus is not sequestered in some protected site within peripheral lympho-

Table 2 Acute IM patients: transformation in mononuclear cell cultures and anti-EB virus titres in sera

Patient no.	Transformation Incidence	Transformation Mean time (weeks)	Serum titre Neutralising	Serum titre Anti-VCA	Serum titre Anti-EBNA
IM 71	4/6	4.5	1:10	1:80	1:10
IM 72	6/6	3.3	1:5	1:80	< 1:2
IM 74	4/6	3.5	1:5	1:80	< 1:2
IM 76	0/6	—	1:20	1:160	< 1:2
IM 92	6/6	4.2	1:20	1:320	1:10
IM 94	2/6	5.0	1:5	1:80	< 1:2
IM 96	6/6	2.8	1:5	1:80	< 1:2

cytes nor is it present as a viraemia, although the latter must obviously occur during prodromal phases before neutralising antibodies develop.

It seems clear therefore that the origin of the EB virus particles present early in cultures of IM mononuclear cells (Table 1, day 3 extracts and ref. 1) and responsible for their transformation to give cell lines, must be peripheral lymphocytes carrying the viral genome in a non-infectious form *in vivo*. These cells when placed *in vitro* do not have the inherent capacity to grow directly into lymphoblastoid lines but are activated into a cycle of virus production; it is this virus which is crucial for the initiation of cell lines since the whole process is inhibited in the presence of neutralising antiserum[1,2]. Peripheral cells in IM must therefore carry EB virus in a different form of infection from that of the malignant cells in Burkitt's lymphoma, where the presence of the viral genome is associated with a ready ability to grow out directly *in vitro* to give a lymphoma cell line[7-9].

It has been suggested[10] that the type of EB viral infection shown by circulating cells in IM may be closer to the latent infections established in ganglia by herpes simplex[11] and varicella-zoster[12] viruses, where the viral genome can remain apparently unexpressed for long periods but can be activated into a cycle of virus production by a variety of stimuli including placing the ganglia in culture[13].

The presence of infectious EB virus in the throat washings of infected individuals[14,15] (whether primary infection was not apparent, or associated with IM) shows that there is a site of virus replication within the oropharynx. It may be that virus-infected lymphocytes leaving this site and entering the circulation have their productive cycle of virus replication repressed in some as yet unexplained way by the combined actions of the cell-mediated and humoral immune responses which are mounted against EB virus-related antigens. Cells containing EB virus genome, scattered through the body's lymphoid tissues *in vivo* and capable of activation to a virus-productive cycle *in vitro*, would explain why lymphoblastoid lines, closely resembling those produced by the *in vitro* transformation of foetal cells but quite distinct from those of Burkitt lymphoma origin[16,17], are obtainable when lymphocytes are cultured from any lymphoma-free individual who is seropositive for EB virus[18].

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Temporary presence of self-reactive cytotoxic T lymphocytes during murine lymphocytic choriomeningitis

MICE injected with lymphocytic choriomeningitis (LCM) virus generate specifically sensitised thymus-derived (T) lymphocytes[1-3]. Such cells have been shown[1,2,4] in *in vitro* ^{51}Cr-release assays to be cytotoxic to target cells infected with LCM virus. The specificity of the cytotoxic reaction has been inferred by the observation that the cytotoxic T lymphocytes (CTL) do not lyse noninfected target cells. Moreover, there is evidence that the activity of the CTLs generated is restricted to those virus infected target cells which are compatible with the CTLs in regard to the major histocompatibility complex (MHC)[5]. This peculiar restriction prompted us to study the specificity of CTLs generated in murine LCM. We observed that 4–6 d after infection with the LCM virus the CTLs generated are specifically cytotoxic to syngeneic target cells, irrespective of whether or not they are infected with LCM virus. On the other hand, as the infection proceeds in time, T-cell mediated cytotoxicity is increasingly restricted to virus-infected syngeneic cells. The results imply that during the early phase after infection, murine LCM may represent an autoimmune phenomenon.

We used strain WE-3 (ref. 6) of the LCM virus. The immunisation regime, the preparation of cells, the characteristics of the cells mediating cytotoxicity and the ^{51}Cr-cytotoxicity assay

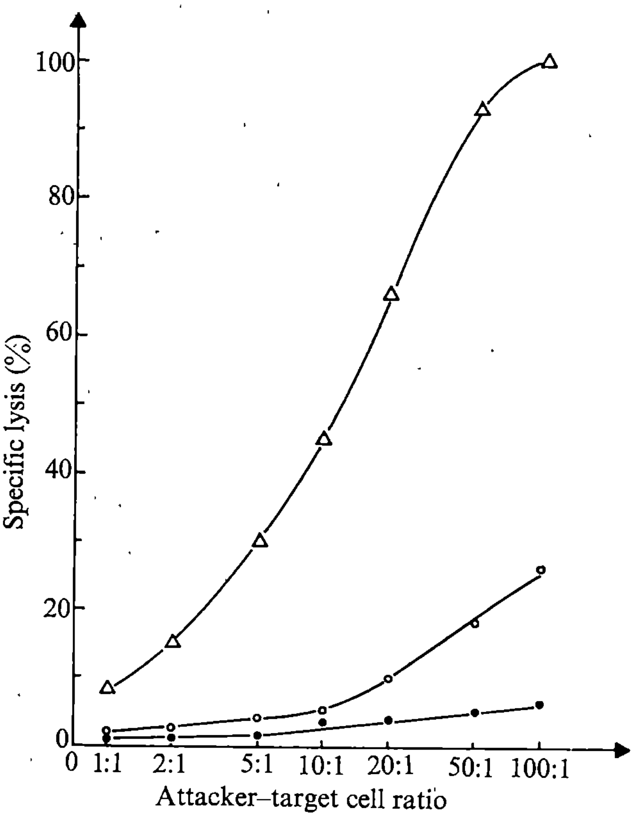

Fig. 1 Dose–response relationship between specific lysis (%) and the ratio of attacker cells to target cells. Spleen cells from LCM infected CBA mice were used at day 8 and tested for cytotoxicity as described in legend to Fig. 2. △——△, Infected L–929 fibroblasts (H–2^k), ○——○, non-infected L–929 fibroblasts (H–2^k); ●——●, non-infected BALB/c embryonal fibroblasts (H–2^d). The spontaneous release of target cells ranged from 20 to 30%. Each point represents the mean of specific lysis (%) obtained at the indicated ratio of attacker cells to target cells. Since in all assays the standard deviation (s.d.) was less than 6%, only the mean is given.

Table 1 Presence of 'self-reactive' CTLs 6 d after infection of CBA mice with LCM virus

	H–2 compatible target cells			Specific lysis (%)	H–2 incompatible target cells		
Experiment no	Cell type	Strain	Virus infected	Non-infected	Cell type	Strain	Non-infected
140	L 929 Fibroblast	C3H	92	44	Embryonal fibroblast	BALB/c	10
142			60	35		BALB/c	5
159			58	73		C57BL/6	23
193			89	43			
159	Embryonal fibroblast	CBA	54	50			
163			52	32		C57BL/6	4
167			57	31		BALB/c	14
158	Macrophage	CBA	17	16	Macrophage	C57BL/6	0
142		B10.A	56	29		BALB/c	5

The cytotoxicity of CBA mice was tested at day 6 after intraperitoneal injection of 1,000 LD_{50} of LCM virus. Each value represents the mean of triplicate determinations obtained at an attacker: target cell ratio of 20:1 (18 h ^{51}Cr-release assay). Standard deviation of the data given was less than 6% Spontaneous ^{51}Cr-release ranged from 18 to 25% for L 929 cells, and from 20 to 35% for fibroblasts and macrophages.

have been described by Pfizenmaier *et al*[7]. In brief, virus-infected or normal target cells (peritoneal macrophages, embryonal fibroblasts or L 929 cells) were labelled with ^{51}Cr-chromate and incubated with immune or normal spleen cells for 18 h. The cytotoxicity assays were performed in triplicates. For each cytotoxic lymphocyte population to be tested, a dose–response curve (Fig 1) was produced. The percentage specific lysis was calculated as described by Wagner[8]. That the detected cytotoxicity was mediated by T lymphocytes is indicated by the fact that pretreatment of the cells with anti-Thy 1.2 serum plus complement abolished their cytotoxic activity.

Splenic T lymphocytes of CBA mice infected intraperitoneally with 1,000 LD_{50} LCM virus of the strain WE-3, developed cytotoxic activity against virus infected H–2 identical fibroblast detectable *in vitro* 4–16 d later. Maximal cytotoxicity was detected at day 7–8. H–2 different (allogeneic) target cells were not lysed (Fig. 2). At the peak of the response (8 d after infection) (Fig. 1), the cytotoxic activity generated seems to have been restricted to virus infected target cells compatible with the attacker cells on either the H–2K or H–2D subregion of the MHC. Virus infected target cells compatible on the I-region of the MHC were not lysed (Table 2). The results are in agreement with the work of Doherty and Zinkernagel[9] and suggest that the target for CTL (as generated at day 8 of LCM) is either closely linked to the phenotypical products coded by the H–2K or H–2D subregion of the MHC, or alternatively, represents serologically defined (SD) 'self' antigens which have been altered in the course of the virus infection[5].

Figure 1 demonstrates that the CTLs generated (at day 8 after infection) are also cytotoxic, albeit much less effectively against non-virus infected H–2 identical target cells. Although this finding was first explained by invoking cross reactivity of the target cells, we studied the specificity of the CTLs in more detail. Surprisingly, we found that CTLs generated during the early phase of LCM were unable to discriminate between virus infected and non-infected H–2 identical target cells; that is, both cell types were lysed equally well (Fig. 2). Yet allogeneic, H–2 incompatible target cells were not lysed above the usual background. Moreover, the existence of 'self-reactive' CTLs seemed to be transient and restricted to the first 3 d of the development of cytotoxic activity. In addition, the data given in Table 1 clearly demonstrate that during the early phase of the LCM disease, the generated CTLs lysed not only the syngeneic L cells which were not infected, but also non-infected syngeneic fibroblasts and macrophages. The results therefore suggested, that the 'self-reactivity' can be detected both with L cells, syngeneic fibroblasts and syngeneic macrophages.

On the other hand, the CTLs obtained at the peak of the

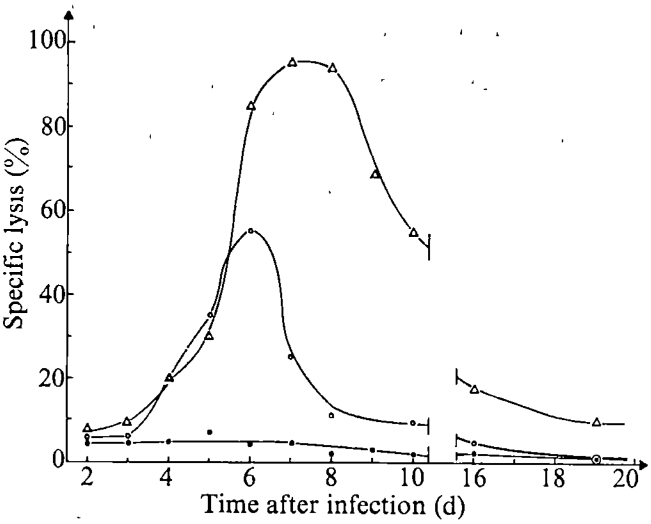

Fig. 2 Kinetics and specificity of cytotoxic T lymphocytes generated during murine LCM. CBA mice were infected intraperitoneally with 1,000 LD_{50} LCM strain WE-3 and at the given time intervals, spleen cells from 3 mice were pooled and tested at different ratios with 5×10^4 target cells in an 18 h ^{51}Cr-release test. Each point represents the mean of specific lysis (%) obtained in triplicate determinations at a ratio of 20 attacker cells to one target cell. Since in all assays the standard deviation (s d.) was less than 6%, only the mean is given. Similar results were obtained in 3 independent experiments. △——△, Infected L-929 fibroblasts (H–2^k); ○——○, non-infected L-929 fibroblasts (H–2^k); ●——●, non-infected BALB/c embryonal fibroblasts (H–2^d). Spontaneous release ranged from 20 to 30%.

Table 2 Specificity of CTLs generated during LCM (8 d after infection)

	Lysis of infected target cells (macrophages) (%)			
Attacker cells derived from*	CBA/J (H–2^k) k kkk k†	DBA/2 (H–2^d) d ddd d	B10 A (4R) (H–2^{h4}) k kbb b	A.TL (H–2^{t1}) s kkk d
CBA/J (H–2^k)	60	7	ND‡	ND
B10.BR (H–2^k)	ND	ND	74	13
BALB/c (H–2^d)	4	45	12	84

*3 mice per group were infected intraperitoneally with 1,000 LD_{50} of LCM virus strain WE-3. After 8 d the spleen cells were tested for cytotoxicity in an 18 h ^{51}Cr-release assay. The results given represent the mean specific lysis (%) obtained in triplicate determinations at a ratio of 20 attacker cells to one target cell. The standard deviation (s.d.) was less than 6%. Lysis of non-infected cells ranged from 3 to 10%. Spontaneous release ranged from 31 to 36%.

†Indicates the haplotype of the MHC, that is, the H–2K, IA, IB, IC, and H–2D subregion, respectively.

‡ND, Not done.

response (day 7–8) were found to be maximally cytotoxic for H–2 identical target cells infected by the virus. At that point in time a very limited amount of anti-'self' cytotoxic reactivity was detected in the spleen of infected mice (Figs 1 and 2). That the cells mediating the anti-'self' cytotoxicity during the early stage of LCM were T cells in type was suggested by the fact that their cytotoxic activity was abolished after treatment with anti-Thy 1.2 serum plus complement (K.P., unpublished).

In conclusion, on the basis of the specificity of the generated CTLs two phases may be distinguished during murine LCM. On the one hand there is an early phase, characterised by the existence of both anti-self-reactive CTLs and by cytotoxic activity against virus-infected, H–2 identical target cells. On the other hand, at the time of maximal cytotoxicity (7–8 d after infection) the CTLs generated exhibit cytotoxic activity restricted against virus-infected, H–2 identical target cells. In control experiments we tested whether the lysis obtained in the case of non-infected target cells was attributable to an LCM-virus infection of the target cells by virus released from the effector cell population. Since, however, addition of LCM-virus to a mixture of effector cells (obtained at day 8) and non-infected syngeneic target cells did not result in lysis of the target cells during the 18 h assay time (K.P., unpublished) that possibility seems to be ruled out. Whether the CTLs generated during the early stage of LCM can be separated into those reactive against virus-infected, H–2 identical cells, and those reactive against self antigens or, alternatively, whether the development of a more stringent restriction of the cytotoxic activity reflects a maturation of the CTLs to be generated, is under investigation.

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Evidence against a dimeric structure for membrane-bound HLA antigens

HUMAN histocompatibility (HLA) antigens are expressed on the surfaces of almost all cell types and according to various criteria are integral membrane glycoproteins[1]. They are composed of equimolar amounts of two dissimilar polypeptide chains which are non-covalently linked and which have molecular weights of about 43,000 and 12,000 as judged by polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS)[2–4]. The larger chain carries the polymorphic allogeneic specificities and all of the carbohydrate[2]. The smaller chain represents β_2-microglobulin, whose amino acid sequence shows marked homology to the C_H3-domain of the γ-1 chain of immunoglobulin G (ref. 5). This homology and other arguments have led to the proposals that HLA antigens and immunoglobulins have a common evolutionary origin[6,7], and that HLA antigens have an immunoglobulin-like structure in which two basic units of one each of the larger and smaller polypeptide chains are linked either non-covalently[8] or by a disulphide bridge[9,10] to give a 4-chain dimer of molecular weight 110,000. In contrast to the latter proposal, some results presented here suggest that HLA antigens do not have a dimeric structure.

HLA activities were measured by inhibition of cytotoxicity[11] using peripheral blood lymphocytes with sera 3002X, P2692B and P3812B for HLA–A2, sera 1209F, 1014I and P1595B for HLA–A1, sera P1422B and P1538B for HLA–B8, and sera P1040B and P3428B for HLA–B13. A highly purified preparation of the plasma membrane of the lymphoblastoid cell-line BRI 8 (HLA type: A1, A2, B8, B13, 4a and 4b) (ref. 1), and of the plasma membrane fraction of a human spleen prepared by a modification of a method described previously[12] (HLA type: A2, A3, BW35) were used as sources of the whole HLA antigens. More than 86% of the plasma membrane HLA activity were solubilised by 1% Na deoxycholate (pH 8.2) as judged after centrifuging at 100,000g for 1 h.

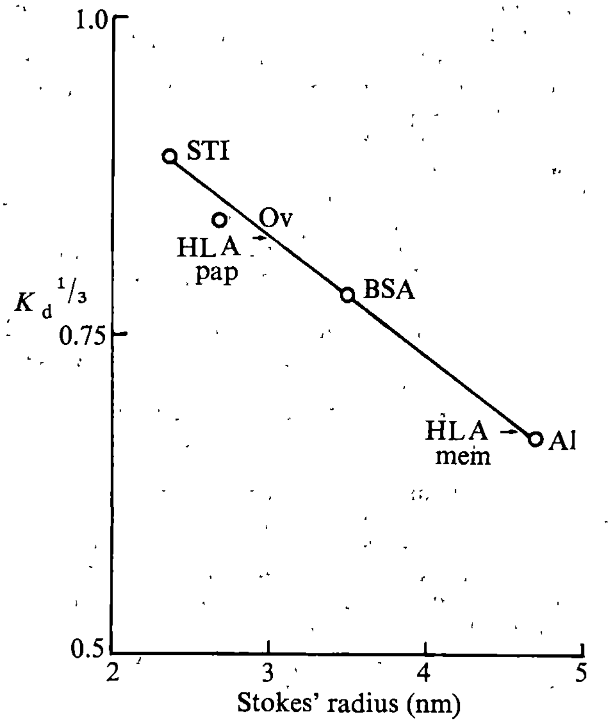

Fig. 1 Relationship between Stokes radii and elution positions ($K_d^{1/3}$) of water-soluble globular proteins from a column of Ultragel AcA 34 in 0.5% sodium deoxycholate. $K_d^{1/3}$ was calculated as previously described[13]. The Stokes radii were those reported by Andrews[14] except for that of soybean trypsin inhibitor which was calculated from the diffusion coefficient and molecular weight[15]. Al, aldolase; BSA, bovine serum albumin; Ov, ovalbumin; STI, soybean trypsin inhibitor. The arrows indicate the measured $K_d^{1/3}$ for the whole HLA antigens (mem) and for the papain-solubilised HLA antigens (pap). Experimental details are given in the legend to Fig. 2.

The molecular nature of HLA antigens was adduced from their gel-filtration behaviour and measurements of their $S_{20,w}$ (ref. 1) and Stokes radii (Fig. 1) in the presence of sodium deoxycholate. Gel filtration of the deoxycholate-solubilised BRI 8 plasma membrane on a column of Ultragel AcA 34 gave (Fig. 2) a single peak of HLA–A2 activity. HLA activities A1, B8 and B13 of the BRI 8 plasma membrane and the HLA antigens of the plasma membrane fraction from the human spleen occupied identical positions with that shown for HLA–A2. Similar elution patterns were also obtained using Sephadex G-150 and G-200, and Sepharose 6B. In each case, the eluted peak accounted for > 86% of the HLA activity added to the column, and no larger aggregates or gross polydispersity was detected. The absence of aggregates in our experiments is in marked contrast with a recent report[10] on a

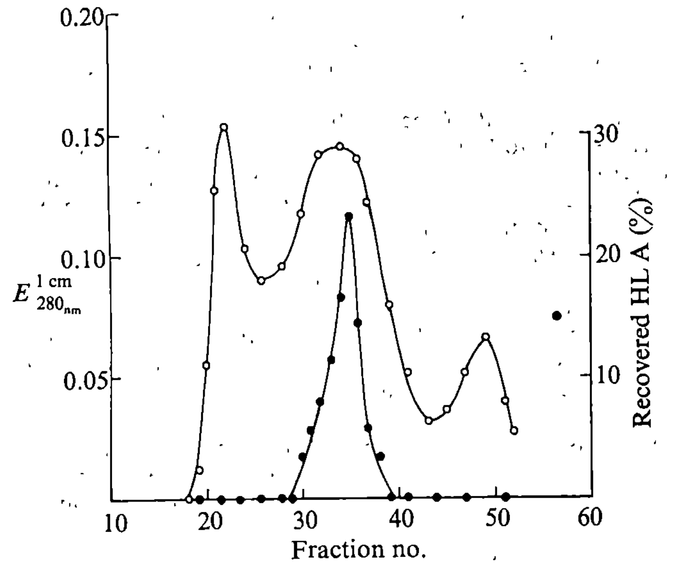

Fig. 2 Gel filtration of deoxycholate-solubilised BRI 8 plasma membrane on Ultragel AcA 34. BRI 8 plasma membrane containing 4 mg of protein was incubated for 30 min at room temperature with 1 ml of 1% sodium deoxycholate in 10 mM Tris-HCl buffer (*p*H 8.2) and centrifuged at 100,000*g* for 1 h. The supernatant (90% of the protein, 86% of the HLA activity) was eluted from a column (91 × 1.6 cm) of Ultragel AcA 34 (LKB Instruments Ltd) with 0.5% sodium deoxycholate at a flow rate of 4.9 ml h^{-1}. Fractions (2.45 ml) were assayed for protein (○; E^{1cm}_{280nm}) and HLA–A2 activity (●). HLA activities A1, B8 and B13 occupied an identical position with that illustrated for HLA–A2. The HLA antigens of the deoxycholate-solubilised plasma membrane from human spleen gave the same elution pattern.

crude membrane preparation from a lymphoblastoid cell line. In that experiment about 40% of the HLA activity was eluted in a second peak, which was provisionally identified as dimer, as well as some larger multimers (see Fig. 3 in ref. 10). The reason for this difference is not known. It is, however, unlikely to be due merely to the use of different cell lines since no aggregates were detected in the current study with human spleen. The main differences between the two studies concern the state of the cells used for membrane preparation and the quality of the membrane fraction. In the present experiments the cells were used immediately after growth (viability > 80%) and the plasma membrane was extensively purified to separate it from endoplasmic reticulum[1]. Furthermore, the HLA antigens were not knowingly subjected to reducing conditions during cell breakage and processing of the plasma membrane. It is possible that Cresswell and Dawson's elution pattern[10] reflects aggregation through disulphide interchange. This aggregation is promoted by storage (compare the elution pattern of an aged sample of human serum albumin[16]). Also, the crude membrane preparations used probably contained endoplasmic reticulum which is rich in an enzyme catalysing sulphydryl–disulphide interchange in various proteins[17].

The molecular sizes and shapes of the HLA antigens in sodium deoxycholate are summarised in Table 1. The values are notable for various reasons. First, the molecular weights of the papain-solubilised HLA antigens in deoxycholate (46,000) agreed closely with those calculated from the values for the β_2-microglobulin and HLA polypeptide chains measured by SDS–polyacrylamide gel electrophoresis (about 12,000 and 34,000, respectively[9]).

Second, the molecular weights of the deoxycholate-solubilised A and B series antigens (88,000 and 78,000 respectively) were larger than those predicted from the sizes of the β_2-microglobulin and HLA polypeptide chains determined by SDS–polyacrylamide gel electrophoresis (about 55,000 including β_2-microglobulin[9]), but were not sufficiently large to accommodate a disulphide-linked dimer. The most likely explanation for this difference is that, as suggested previously[1], HLA antigens resemble other integral membrane proteins (for example cytochrome b_5; ref. 18) in possessing a hydrophobic domain which binds non-ionic and weakly anionic detergents[19,20]. As a result the deoxycholate-solubilised HLA antigens will possess anomalously high molecular weights when assessed relative to water-soluble globular proteins. This anomaly will not, however, apply to estimates of the molecular sizes of the papain-solubilised antigens, because of the removal of the hydrophobic domain during proteolysis and the consequent loss of detergent binding. If it is assumed that the true molecular weight of HLA antigens is 55,000 (as determined by polyacrylamide gel electrophoresis[9]) and that the difference between this molecular weight and those reported for the whole antigens in Table 1 is entirely due to bound detergent, then the A and B series antigens bind 0.57 and 0.39 g of deoxycholate per g of protein respectively. These values are in accord with those reported for various membrane proteins (0.29–0.64 g per g of protein)[20] and with those estimated by equilibrium dialysis for two fractions of BRI 8 plasma membrane containing about 3 and 15% of HLA antigens (0.68–0.72 g per g of protein; D. Snary, unpublished). Detergent binding is also consistent with the higher molecular weights reported for HLA and H–2 antigens (460,000 and 380,000, respectively) in different non-ionic detergents (Brij 99 and Nonidet P40 respectively)[3,21], since these probably express the higher micelle sizes of the bound detergent.

Alternatively, the larger molecular weight of the deoxycholate-solubilised HLA antigens could accommodate a non-covalently bonded dimer[8] if deoxycholate caused dissociation. This is considered improbable primarily because comparisons of the gel-filtration patterns of haemoglobin on Sephadex G-200 in the presence and absence of 1% Na deoxycholate failed to reveal any evidence for dissociation. This result is especially significant since haemoglobin is already partly dissociated in aqueous solution and the majority of the interactions between the α_1 and β_2 subunits are non-polar[22] and would be expected to be particularly sensitive to dissociation by detergent.

Third, the apparent difference between the molecular weights of the deoxycholate-solubilised A and B series antigens (Table 1) probably arises from a larger amount of deoxycholate binding to the A antigens than to the B antigens. This variation may reflect a difference in hydrophobicity in which case it may indicate that the A series antigens are inserted more deeply into the lipid bilayer than B series antigens.

Fourth, estimates of the frictional ratios[13] of the A and B antigens gave 1.49 and 1.55 respectively. Although these values suggest that the membrane-bound antigens are moderately

Table 1 Molecular size and shape of HLA antigens in sodium deoxycholate

HLA antigens	Antigen series	$S_{20,w}$	Stokes radius (nm)	Molecular weight	f/f_0
Na deoxycholate-solubilised	A	5.15	44.0	88,000	1.49
	B	4.55	44.0	78,000	1.55
Papain-solubilised	A, B	3.68	29.8	46,000	1.26

$S_{20,w}$ values were determined previously by sucrose density gradient centrifugation in 1% sodium deoxycholate (see Table 3 in ref. 1). The Stokes radii were measured by gel filtration on a column of Ultragel AcA 34 that had been standardised using water-soluble globular proteins in 0.5% sodium deoxycholate (Fig. 1). Molecular weights were calculated from $S_{20,w}$ and Stokes radii (α) by using the relationship molecular weight = $6\pi N\alpha\eta S/1-\rho\bar{v}$ (ref. 13), where N is Avogadro's number, η the viscosity and ρ the density of water at 20 °C. The partial specific volume ($\bar{v}$) for the papain-solubilised HLA antigens was taken to be 0 72, whereas that for the deoxycholate-solubilised whole antigens was assumed to be 0.74 on the basis that they contain 8% carbohydrate (E. Appella, personal communication) and bind 0 5 g of deoxycholate per g of protein (see text). The frictional ratio (f/f_0) was calculated according to ref. 13.

asymmetrical, the possibility that the asymmetry is primarily due to the association of the bound detergent with one region of the molecule only cannot be ruled out at present.

In contrast to previous work[9,10], no evidence was obtained in the current study for the presence of dimers. It was concluded that the above results cast doubt on the validity of the 4-chain, dimeric, immunoglobulin-like model that has been proposed for membrane-bound HLA antigens, but do not rule out the possibility of a common evolutionary origin for HLA antigens and immunoglobulin.

We thank Dr A. F. Williams for the gel-filtration analysis of HLA antigens on Sephadex G-200. The water-soluble fragments of HLA antigens (type: A1, A2, B7 and B12) produced by papain digestion were donated by Dr A. R. Sanderson. This work was partly supported by an MRC grant (to the Genetics Laboratory, Oxford). P.G. is a recipient of an MRC research scholarship.

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Identification of Ss protein as murine C4

DEMANT *et al.*[1] showed that the Ss-Slp region in the H–2 complex in mice affected the level of serum complement, the Ss high mouse having a higher complement activity than the Ss low mouse. They went on to show that antiserum to Ss protein could deplete complement activity from mouse serum, which has been confirmed in another laboratory[2]. This was the first association described between complement and the principal histocompatibility locus. Further associations have since come to light. In the mouse, C3 levels are controlled in part by a gene in the H–2 region[3] although the effect is not large On the other hand, the 'C5 deficiency gene' in the mouse is not linked to H–2 (ref. 4). In man the allotypes of factor B show close linkage to HLA (ref. 5). C2 deficiency in man is also HLA linked in family studies[6] and a marked linkage disequilibrium has been observed between the 'C2 deficiency gene' and the haplotype HLA10,W18. In the family described with C4 deficiency, HLA linkage has also been described[7]. The C3 allotypes in man, however, are not linked to HLA, nor is the 'C3 deficiency gene'[8]. For guinea pigs, the 'C4 deficiency gene' is linked to the principal histocompatibility locus[9]. Démant *et al.*[1] showed that the Ss protein is likely to be a complement component, and in this communication we provide evidence that the Ss protein is the murine equivalent of the fourth component, C4.

Antiserum to Ss was prepared and rendered monospecific as before[1]. Antiserum to mouse C3 was prepared as before[10] and rendered substantially monospecific by absorption with the serum of mice treated with cobra venom factor. A test of total haemolytic activity confirmed the observation that the Ss high mice had almost twice the activity seen in the Ss low mice.

Functional tests for the components that can be measured by standard functional assays for human complement[11] were undertaken as shown in Table 1. These results show several components which the Ss protein cannot be. Thus Ss is unlikely to be any of the components C5–C9 since the lytic titre of mouse serum on erythrocyte antibody complexes which reacted with the first four components of complement (EAC 1423) is somewhat higher in the serum of the Ss low mice. Similarly, the Ss protein seems not to be C1 nor either of the two factors of the alternative pathway which are required for reaction with cobra venom factor, that is factor B or factor D. Since human factor B is known to be linked to the HLA locus, the observation that the Ss protein seems not to be factor B is particularly significant The observation was confirmed by showing that anti-Ss failed to prevent the diffusion of the cobra venom C3-convertase formed on adding cobra venom factor to normal mouse serum. Depletion of factor B does not in any case decrease the total haemolytic titre of human serum (A.M. and P.J.L., unpublished). Functional tests were also carried out for the C$\bar{1}$ inhibitor and the C3b inactivator. In neither case were differences found between Ss high and Ss low mice.

Table 1 Functional complement activity in Ss high and Ss low strains of mice

	B 10.D2/N H–2^d Ss high (h-a)	B10.BR H–2^k Ss low (l-o)
CH50 per ml	8	4
C1 (titre)	1,280	1,280
C$\underline{5}$ ~ C9 (titre)	3	8
C$\underline{1}$-inhibitor (C$\bar{1}$ required to react with 100 µl serum)	20 µl	30 µl
C3b-inhibitor (titre) (measured by conglutinogen activation)	40	40
CVF-cofactors (taken as) (factors B + D)	100%	98%
C3 (antigenically)	158	180

CVF, Cobra venom factor. h-a, Ss $^{\text{high}}$ Slp^d; l-o, Ss $^{\text{low}}$ Slp^0.

Functional tests for C4, C2 and C3 were difficult to carry out with mouse serum. C3, however, could readily be measured immunochemically and, insofar as a difference was discovered among the mice tested, there was a modest increase in C3 levels in the Ss low mice. This agrees with the findings of Ferreira and Nussenzweig[3]. To attempt to identify Ss protein as either of the two remaining components—C4 and C2—experiments were carried out using an anti-Ss antiserum to detect Ss protein as a fixed complement component on erythrocytes (Table 2). When fresh mouse serum was added to EA, an intermediate was made that could be agglutinated more strongly and to higher titre with anti-Ss showing that more Ss protein has been fixed. In this experiment a C5-deficient mouse serum was also used to make EAC. This intermediate also was agglutinated (albeit weakly being a Ss low strain) by anti-Ss serum. This confirms that Ss is not a component of the complement sequence at the C5 stage or beyond.

When the EAC made with the Ss high serum was allowed to decay, either in EDTA to remove C1 as well as C2, or in half-ionic strength complement buffer to remove C2 only,

Table 2 Assessment of Ss as agglutinogen on complement-coated cells

	EAC(CBA)	EAC(AKR)	EAC(BALB/c)	EA
Ss status	l-o	l-o	h-a	
C5 status	+ve	−ve	+ve	
Agglutination titre with				
Anti-C3	729	729	729	0
Anti-Ss	1	27 (weak)	27 (strong)	0

				Agglutination titre with anti-Ss
EAC (BALB/C)		Undecayed	(EAC1423(5))	27
EAC (BALB/C)		Decayed 90 min in CFT–sucrose	(EAC143)	27
EAC (BALB/C)		Decayed 90 min in EDTA–saline	(EAC43)	27
			EA	0
EA	+	NH_3 treated BALB/c complement		0
EAC (CBA)	+	NH_3 treated BALB/c complement		1
EA	+	Heated (56 °C, 30 min) BALB/c complement		0

The figures are the highest dilutions of antiserum giving rise to agglutination. CFT, Complement fixation test buffer.

agglutination by anti-Ss was not impaired. This strongly suggests that the Ss protein is C4. A further experiment was performed to confirm this conclusion. EAC was made with Ss low serum and subsequently incubated with NH_3-treated Ss high serum. This conferred on the intermediate no further reactivity with anti-Ss This experiment indicates that Ss is not C2. The same experiment can also be taken as evidence that Ss is not properdin because activated properdin will attach to fixed C3b (ref. 12).

Table 3 Assessment of Ss by haemagglutination inhibition

Strain		Ss status	Dilution of serum inhibiting agglutination of EAC (BALB/c) by 3 agglutinating doses of anti-Ss
C3H/He/j	♂	l-o	20
CSW	♂	h-a	140
B10.A	♂	h-a	140
B10.D2/n	♂	h-a	240
B10.BR	♂	l-o	20
BALB/c	♂	h-a	140
BALB/c	♀	h-a	80
CBA	♀	l-o	30

Using EAC made with the Ss high serum and three minimum haemagglutinating doses of anti-Ss serum, we could assay the Ss level of several mouse sera by haemagglutination inhibition. Although such experiments are complicated by the presence of immunoconglutinins in individual mouse sera, Table 3 shows that it is possible to get a measure of the concentration of Ss in serum by this technique. This would confirm that the antigen detected by the anti-Ss serum on EAC is the same as that normally measured by precipitation.

These experiments show that the protein reacting with anti-Ss antiserum is a complement component bound firmly and stably to EA at a stage before C5 and that the properties of the Ss 'agglutinogen' correspond to what is anticipated for C4.

In view of the probability that the structural genes for several complement components are coded in the H–2 region, the possibility should not be wholly discounted that the method used to raise anti-Ss antisera could raise antibodies to more than one complement protein. It might therefore be preferable to say that murine C4 is an Ss protein rather than necessarily the unique Ss protein.

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H–2 linked Ss protein is C4 component of complement

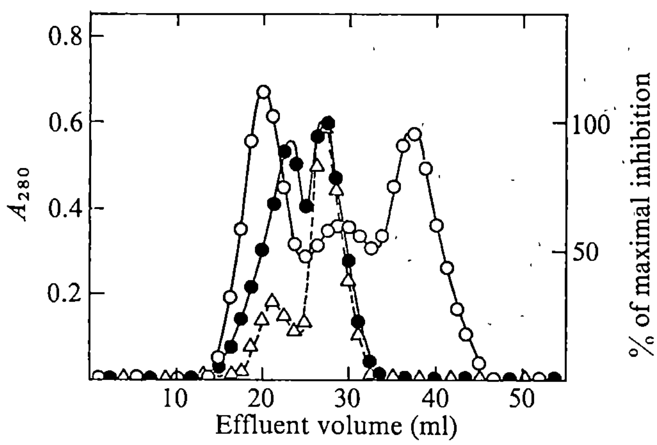
THE major histocompatibility complex in the mouse encompasses several distinct loci, which, with one exception, control the expression of cell surface glycoproteins. The exception is the Ss-Slp traits[1]. The Ss trait involves regulation of the level of a plasma protein, the Ss protein[2]. The S region of the H–2 complex, whose marker gene controls the Ss-protein concentration, also regulates the haemolytic complement level[3]. This has led to the suggestion that the Ss protein is a component of complement[3,4]. We have isolated the Ss protein and demonstrate here that it is the murine equivalent of C4.

In our initial attempts to purify the Ss protein we used conventional fractionation procedures. Serum from male BALB/c mice (*Ss*^{h} genotype) was initially separated by Sephadex G-200 gel chromatography followed by successive steps of DEAE–Sephadex ion-exchange chromatography (twice), and as the final purification step, recycling chromatography on Sephadex G-200. Ss protein was monitored in chromatographic fractions by immunodiffusion analyses, using a rabbit antiserum against a high molecular weight plasma protein fraction from BALB/c serum. The antiserum was rendered monospecific by repeated absorptions with serum from CBA (Ss^l phenotype) male mice[2]. The isolated protein was homogeneous and of high purity as shown by several physicochemical, chemical and immunological criteria (L.S., in preparation). It consisted of four polypeptide chains, each of apparent molecular weight about 35,000, which were held together in pairs by disulphide bonds. But during the isolation procedure the molecular weight of Ss protein became considerably smaller. Sedimentation–diffusion analyses of the Ss protein present in unfractioned plasma suggested a molecular weight of about 200,000 compared with the 140,000-dalton molecule isolated. It thus seemed likely that either the Ss protein in plasma is linked to another protein, which was lost during isolation, or is the Ss protein proteolytically degraded during isolation. We therefore tried to improve the isolation by speeding it up. A rabbit antiserum was raised against the 140,000-dalton component. This antiserum reacted with a single component in plasma, as shown by extensive immunodiffusion and immunoelectrophoretic analyses. A radioimmunoassay developed with this antiserum (L.S., in preparation) showed that male mice contained about twice as much of the Ss protein as did female mice, regardless of strain. Furthermore, strains with the Ss^h haplotype had approximately 10 times more of the isolated component in serum than mice of the Ss^1 genotype. These data strongly support the view that the antiserum is directed against the Ss protein[1].

The IgG fraction of the anti-Ss-protein serum was coupled covalently to Sepharose 4-B to obtain an immunosorbent column. The Ss protein was isolated from EDTA-plasma, which had first been passed over a column of Sepharose 4-B coupled with aggregated rabbit IgG to remove material sticking nonspecifically to IgG. The breakthrough peak recovered from the aggregated Ig column, which contained all the immunologically active Ss protein as measured by radioimmunoassay, was subsequently passed over the anti-Ss column. The bound material, which was desorbed by lowering the *p*H of the eluent, was labelled with ^{125}I and analysed by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis and by gel chromatography in 6 M guanidine hydrochloride. A single component with an apparent molecular weight of slightly less than 200,000 was obtained from the anti-Ss column. On reduction and alkylation in denaturing conditions, the Ss protein resolved into three (or sometimes four) distinct polypeptide chains. Two of the chains appeared in the 70,000–80,000-dalton region on a column equilibrated with 6 M guanidine hydrochloride. The third chain had an apparent molecular weight of about 23,000 and in some preparations small amounts of a 13,000-dalton component was present. It is thus reasonable to conclude that the Ss protein isolated by conventional techniques most likely represents a proteolytically derived fragment of the larger molecule.

The molecular characteristics of the immunosorbent-purified protein suggested that if indeed it is a component of complement it most likely is C3, C4 or C5, on the reasonable assumption that mouse and human complement components are similar in structure[5]. Since mouse strains genetically deficient in C5 may have the Ss^h genotype[6], C5 could be ruled out as the Ss protein. The level of C3 was estimated by single radial immunodiffusion of serum from three strains with the Ss^l genotype and five with the Ss^h genotype. No statistically significant difference in the serum concentration of C3 was found. Although it has been claimed that the level of C3 is regulated in part by the H–2 complex, even in that study no correlation was found between the *Ss* genotype and the level of C3 (ref. 7).

Fig. 1 Gel chromatography of BALB/c male serum on a Sephadex G-200 column (76×1 cm) equilibrated with 0 02 M Tris-HCl buffer, *p*H 8.0, containing 0.15 M NaCl. Total protein was estimated by absorbance at 280 nm (○). The distribution in the effluent of Ss-protein-reacting material was assessed by radioimmunoassay with the 140,000-dalton fragments of the Ss protein as the ^{125}I-labelled component and with antiserum against the Ss protein (●) or human C4 (△) as the binding reagent.



Our efforts were therefore concentrated on the possible relationship between C4 and the Ss protein. Human C4 was isolated[8] and its molecular characteristics were compared with those of the Ss protein. The human C4 had a molecular weight on SDS–polyacrylamide gel electrophoresis very similar, if not identical, to that of the immunosorbent-purified Ss protein. When human C4 is reduced and alkylated two large polypeptide chains are released. Their molecular weights were indistinguishable from those of the main Ss-protein chains. Furthermore human C4 contained a polypeptide chain with an apparent molecular weight of 35,000 in contrast to the 23,000-dalton component derived from the Ss protein. This discrepancy in the polypeptide chain structure between the two proteins may be genuine, but could have arisen because different isolation procedures were used. It should be remembered that serum contains a C4b inactivator which sequentially cleaves C4 into smaller fragments[9].

To examine further the relationship between human C4 and the Ss protein, the possibility of immunological cross reactivity was examined. A sensitive radioimmunoassay for human C4 was developed since in immunodiffusion analyses anti-Ss did not precipitate human C4 and vice versa. It could however, be shown that the binding of ^{125}I-labelled C4 to a monospecific antiserum against human C4 was almost abolished in the presence of immunosorbent-purified Ss protein. In the Ss protein radioimmunoassay human C4 competed very efficiently with ^{125}I-labelled Ss protein for binding to the antibodies. The specificity of the reactions was examined by monitoring the occurrence of the Ss protein in fractions obtained after gel chromatography of mouse serum on a column of Sephadex G-200 Figure 1 shows two peaks of Ss-protein-reacting material produced in the Ss protein radioimmunoassay. The same distribution of material was obtained when antibodies against human C4 were used, and similar patterns were recorded even when ^{125}I-labelled human C4 was used. These data strongly suggest that Ss protein is murine C4. The earliest eluted Ss-protein-containing peak is greatly diminished, with a concomitant increase in the amount of Ss protein present in the second peak, in EDTA-plasma. The high molecular weight of the Ss-protein-reacting material in the first peak (sedimentation coefficient about 18S) may represent Ss protein bound to other components, especially since it is known that in fluid phase C423 may occur[10].

To identify the Ss protein unequivocally it must be shown to reconstitute functionally a C4-deficient serum (R4-serum) We have so far failed to do this. The drastic elution conditions that have to be used to desorb the Ss protein from the immunosorbent column may derange the protein so that when it is recovered it is inactive. We were able, however, to test whether Ss-depleted serum was deficient in C4. Figure 2 shows that the material that passed over the anti-Ig column had substantial C4 activity whereas the Ss-protein-depleted fraction was devoid of functional C4 (Fig. 2). This seems to identify the Ss protein functionally with C4.

Lachmann *et al.* in the preceding communication[11] examined the relationship between the *Ss* genotype and various complement components in functional complement assays. Using an antiserum rendered specific for Ss protein, they assessed by absorption the presence of Ss on cells coated with complement. Although using a different

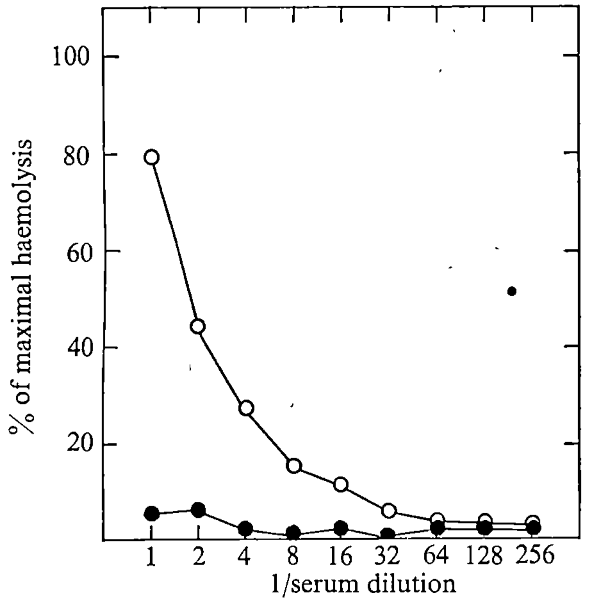


Fig. 2 Reconstitution of the haemolytic complement activity of C4-deficient guinea pig serum with Ig (○) or Ss protein (●) depleted BALB/c male EDTA-plasma. Aliquots of the mouse plasma were depleted of Ig and Ss protein, respectively, by passage over immunosorbent columns containing covalently bound antibodies of the appropriate specificities. The breakthrough peaks recovered from the immunosorbent fractionations were concentrated separately, dialysed against veronal buffer, and adjusted to the original volume of the plasma sample. Guinea pig serum was depleted of C4 by NH_3 treatment (R4 serum). EA was obtained by mixing sheep red blood cells (SRBC) with the IgM fraction of a rat antiserum against SRBC. Haemolytic complement assay was performed as follows. To 100 µl of R4 serum, diluted 1/20 in gelatin-containing veronal buffer, 100 µl of the relevant mouse plasma fractions, depleted of Ig or Ss protein, at the indicated dilutions, and 50 µl of 1% EA were added. After incubation at 37 °C for 30 min, the mixtures were diluted with 1 ml of cold veronal buffer. The cells were pelleted by centrifugation at 400*g* for 10 min and the absorbance at 415 nm in the supernatant was estimated. The 100% value in the figure is taken as the absorbance obtained in the complement assay with untreated BALB/c EDTA-plasma. For all values blank readings have been subtracted.

approach than the present, their study demonstrates that the Ss-protein is murine C4.

Our data reinforce the importance of the H-2 region in various immunobiological systems. As well as its role in cell mediated immunity, the major histocompatibility complex now seems to be involved in an effector function of the humoral immunity system. Not only C4 but C2 (ref. 12), C3 (ref. 7) and factor B of the alternate pathway[13] are regulated by this gene complex. Whether it also controls other components of the complement system remains to be elucidated. We thank Dr P. J. Lachmann for communicating his data before publication. This work was supported by grants from the Swedish Medical Research Council and the Swedish Cancer Society.

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Influence of negative lipids on interactions between artificial membranes and cholinergic drugs

EFFORTS have been made to incorporate "cholinergic systems" into artificial lipid membranes[1–5]. In all cases, material was obtained from animal tissues and incorporated into artificial systems, cholinergic drugs were added to the bathing solutions and different "permeability changes" were described. The available information on the interactions between pure lipid membrane and cholinergic agents is, however, scarce[6] and to establish the role of proteins and lipids in the reported findings is difficult. We report here results showing that a negatively charged lipid induces "cholinergic reactivity" in artificial lipid membranes which can be prevented by *d*-tubocurarine.

Planar artificial lipid membranes were made in a septum separating two aqueous phases. Increasing voltages were applied from an external d.c. source and the membrane resistance was calculated from the current–voltage relationship which was constant between 0 and 150 mV. Different salt gradients were then established across the membrane

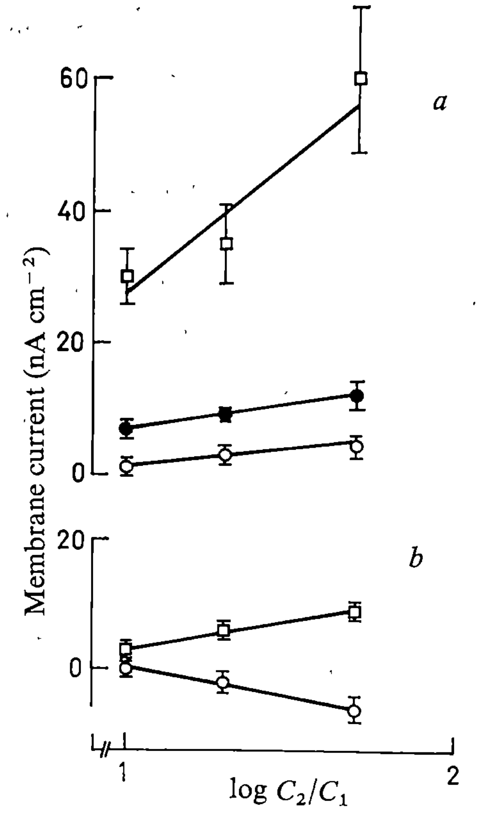

Fig. 1 Membrane current (voltage clamped at 0 mV) as a function of AChCl gradients across the membrane. *a*, Control; *b*, in the presence of *d*-TCCl (10^{-4}M) in the bath. ○, PC plus cholesterol membranes; ●, PC, cholesterol and 1% PS; □, PC, cholesterol + 10% PS.

maintaining the potential clamped at 0 mV. The current flowing through the membrane was recorded and the corresponding membrane potential was calculated using the previously determined resistance. A detailed description of this technique was made elsewhere[7]. The membrane-forming solutions contained 2.3 mg ml^{-1} synthetic DL-α-dipalmytoyl lecithin (PC) (Sigma, standard for chromatography), 2.3 mg ml^{-1} synthetic cholesterol (Sigma, 99%) and, in some cases, different amounts of bovine phosphatidyl-L-serine (PS) (Sigma, 95%) dissolved in chloroform–methanol–tetradecane (1.0 : 0.8 : 0.5; v/v).

Table 1 Calculated potentials for a tenfold salt gradient across the membrane (mV)

	100% PC	99% PC–1% PS	90% PC–10% PS
AchCl	−1.6±2.0	−8.4±1.1	−20.2±1.3
ChCl	−1.2±0.6	−4.5±0.4	−17.9±1.4
d-TCCl	−8.6±2.1	−13.6±2.8	−33.4±6.1
NaCl	+12.6±3.5	+7.4±1.2	−30.1±2.8

The negative sign indicates cationic selectivity and the positive sign indicates anionic selectivity. Membrane potentials were calculated from membrane current and membrane resistance.

Figure 1*a* shows the current that flows through the membrane when different acetylcholine chloride (AChCl) (Sigma, preweighed vials, 99%) gradients were established. The membranes were prepared in the presence of 1 mM AChCl solution buffered with 1 mM Tris-PO$_4$ (*p*H 7, at room temperature). It can be observed that current intensity is a linear function of the logarithm of the ratio of AChCl concentration in the two chambers. The corresponding membrane potentials are shown in Table 1. The membrane solution used contained as amphipathic molecule only PC, PC plus 1% PS or PC plus 10% PS. It can be observed that as PS concentration increases, membrane current and membrane potential become greater. The sign of the potential indicates cationic selectivity. These results show that, specially in the presence of negative lipids, AChCl gradients at high concentrations can induce cationic membrane potentials showing Nernst-type behaviour. This AChCl potential can be blocked by *d*-tubocurarine (*d*-TCCl). Membranes were made in the presence of 10^{-4} M *d*-TCCl (Sigma) and different AChCl gradients were applied. Figures 1*a* and *b* compare results with membranes containing only PC or PC plus 10% PS with or without *d*-TCCl in the bath.

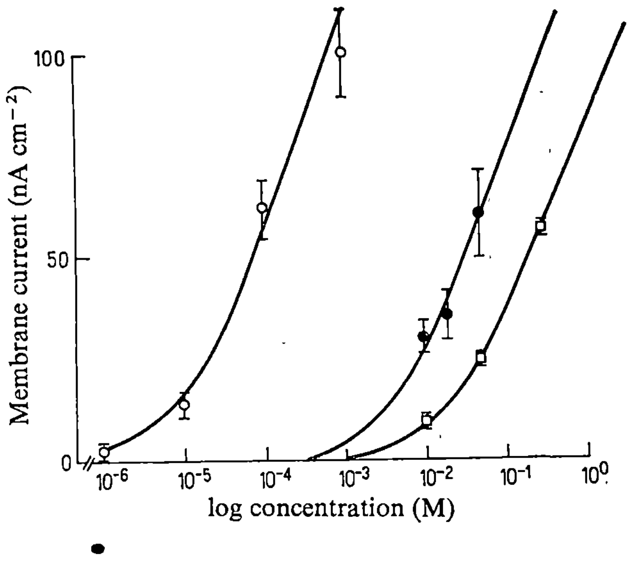

Fig. 2 Membrane current (voltage clamped at 0 mV) as a function of the concentrations of different agents on one side. ○, *d*-TCCl; ●, AChCl; □, NaCl. The membranes were initially made in 1 mM Tris-PO$_4$ buffer. The *d*-TCCl curve was drawn according to the experimental points. The AChCl and NaCl curves were obtained by moving the *d*-TCCl curve to the right.

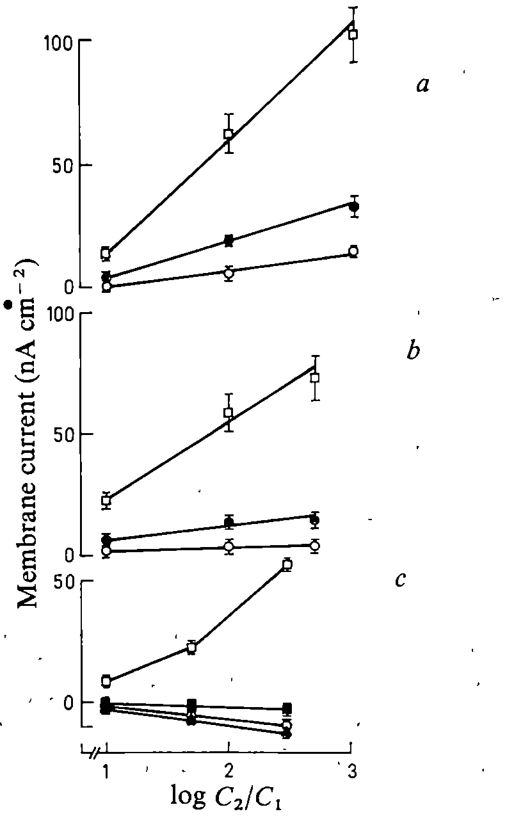

Fig. 3 Membrane current (voltage clamped at 0 mV) as a function of different gradients. ○, PC + cholesterol membranes; ●, PC, cholesterol and 1% PS; □, PC, cholesterol and 10% PS; ▲, PC + cholesterol in the presence of *d*-TCCl (10^{-4}M); ■, PC, cholesterol and 10% PS in the presence of *d*-TCCl (10^{-4}M). *a*, *d*-TCCl gradients; *b*, ChCl gradients; *c*, NaCl gradients.

To interpret these results AChCl, *d*-TCCl and NaCl gradients were established across membranes containing different proportions of PS. Membranes were made in 1 mM Tris-PO$_4$ (*p*H 7.0) and the tested salts were added to one side of the membrane at increasing concentrations. Figure 2 shows the results obtained. In the presence of 10% PS NaCl, AChCl or *d*-TCCl induce cationic potentials. The concentration ranges in which this phenomenon is apparent are, however, quite different. These results could be explained by a different effective concentration for each one of these species at the membrane interface. Assuming that *d*-TCCl accumulates at the surface of the membrane to a larger extent than either AChCl or NaCl, its blocking effect becomes predictable.

The membrane potentials induced by the presence of *d*-TCCl gradients are also sensitive to PS concentrations (Fig. 3*a*; Table 1) and it can be remarked that in the case of *d*-TCCl even in the presence of an amphoteric phospholipid (PC) a clear cationic selectivity was observed.

In a previous work[8] a difference between choline chloride (ChCl) and AChCl responses was reported when natural extracts were used. This is not the case in this system. Figure 3b and Table 1 indicate that the behaviour in the presence of ChCl is not distinguishable from the behaviour when AChCl is tested.

It has been reported that the membrane potential developed when a NaCl gradient is established across undoped membranes is a function of the net charge of the lipids[9] used. We have shown that a low percentage of PS is sufficient to develop cationic selectivity (Figure 3*c*), although, in practice, pure PC membranes did not discriminate between cations and anions.

The present results show that pure lipid membranes containing a small amount of negative lipids can show cationic selectivity. Furthermore, when the salt gradients are established with cholinergic agents, membrane potentials occur which also show cationic selectivity. The cationic selectivity could be due to a higher permeability for these organic cations when negative lipids are present. Adsorption potentials probably arise, however, at the membrane interface and, consequently, it is not possible to know whether or not a local equilibrium exists at the interface. If the calculated potentials are equal to a distribution potential, or an adsorption potential cannot be established in our experimental conditions[10], these potentials would be similar to those described by Bean *et al.*[11] when procaine or other organic molecule gradients were applied across membranes containing brain lipids (whose proportion of negative lipids was not reported).

These types of interactions could be the cause, at least in some cases, of the "cholinergic responses" obtained with membranes containing natural extracts. This is especially true in the case of *d*-TCCl, generally used at concentrations similar to those used here[2,4,5]. It has been reported that proteolipids could have an important role in cholinergic systems[12] and it is also known that these molecules are rich in negative lipids. When AChCl is applied on artificial membranes containing proteolipids from electric eel a "conductance change" appears, which can be abolished by *d*-TCCl[8]. In view of our results the role of membrane potentials due to the presence of negative lipids must be taken into account when interpreting these type of experiments. Similarly, the *d*-TCCl-dependent membrane potential observed when salt gradients are established across proteolipid containing membranes[7] could be also dependent on the presence of negative lipids. We can speculate that proteolipids induce negatively charged clusters in the membrane that favour the adsorption of different cations. The role of the interactions described here in the pre- or post-synaptic systems is a matter of discussion.

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Competition for sulphate during detoxification in the gut wall

ALTHOUGH the absorptive function of intestinal cells has been studied in depth, the capacity of intestinal mucosa to metabolise ingested substances has received less attention. But the gut wall presents a first line of defence against potentially harmful agents and it is likely that its detoxifying properties are important.

Gut cells are capable of oxidative, reductive, hydrolytic and various synthetic reactions, including acetylation and conjugation with sulphate and glucuronic acid[1]. There are relatively few quantitative data on drug metabolism within the gut mucosa but Conolly *et al*[2] demonstrated that the β agonist isoprenaline, administered orally, is excreted principally in the urine as an ethereal sulphate, and their experiments suggested that conjugation took place within the gut wall. Intravenous isoprenaline was excreted in the urine largely unchanged. Sulphate conjugation greatly reduces the systemic availability of isoprenaline which can produce pharmacological effects in μg quantities intravenously[3], but which is used in mg amounts as an oral drug[4]. George, *et al.*[5] showed that when isoprenaline is placed in an isolated dog gut loop *in situ*, only a small fraction appears as free isoprenaline in the venous effluent draining the loop, the major proportion being a sulphate conjugate. They also demonstrated that inclusion of salicylamide with isoprenaline in the loop resulted in greatly increased concentrations of free isoprenaline in the venous effluent. Salicylamide is also metabolised by sulphate conjugation[6], and their findings suggested that competition for available sulphate in gut wall enabled more free isoprenaline to drain from the gut. We have investigated this possibility in the intact animal.

Seven female greyhound dogs (20–34 kg) were placed in a restraining stand and remained conscious throughout the experiment. Heart rate was recorded by electrocardiograph. On a control day, after heart rate had stabilised, each dog was given isoprenaline, 3.0 mg base in 200 ml of water by stomach tube. Four dogs also received ^{3}H-isoprenaline (+Isoprenaline-(7^{3}H)-HCl, 1.8 Ci mg^{-1}, Radiochemical Centre, Amersham) and their plasma isoprenaline was measured[2] in systemic venous blood. Heart rate was recorded for 180 min On a test day, atfer approximately 10 d, the experiment was repeated except that 600 mg of salicylamide was given with isoprenaline. Five of the dogs also received 600 mg of salicylamide in gelatin capsules by mouth 2 h and 1 h before isoprenaline and salicylamide. Three of the dogs were later given 600 mg of salicylamide and 1.0 g of L-cysteine by mouth 2 h and 1 h before receiving a solution containing 1.0 g of L-cysteine, isoprenaline and salicylamide, by stomach tube.

When isoprenaline alone was given, the mean peak plasma isoprenaline concentration was 2.02±0.28 (s.e.m.) ng ml^{-1} and the area under the plasma isoprenaline–time curve was 211.7±32.2 ng ml^{-1} min. After isoprenaline had been given with salicylamide, peak plasma concentration

Table 1 Peak heart rate responses (beats min^{-1}) and areas under heart rate–time curves (beat min^{-1}) in seven conscious dogs given isoprenaline (3.0 mg base), salicylamide (600 mg) or L-cysteine (1.0 g) by mouth as indicated

Dog		Isoprenaline alone	Isoprenaline+ salicylamide	Isoprenaline+ salicylamide+ L-cysteine
A	Peak	56	86	
	Area	2,355	4,889	
B	Peak	52	134	
	Area	2,143	6,082	
C	Peak	70	108	
	Area	2,529	4,719	
D	Peak	45	125	
	Area	1,430	4,435	
E	Peak	74	106	84
	Area	1,468	4,220	2,668
F	Peak	71	151	52
	Area	1,736	3,678	1,888
G	Peak	92	142	111
	Area	3,182	4,420	2,982
Mean	Peak	65.7	121.7	82.3
	Area	2,120.4	4,634.7	2,512.6

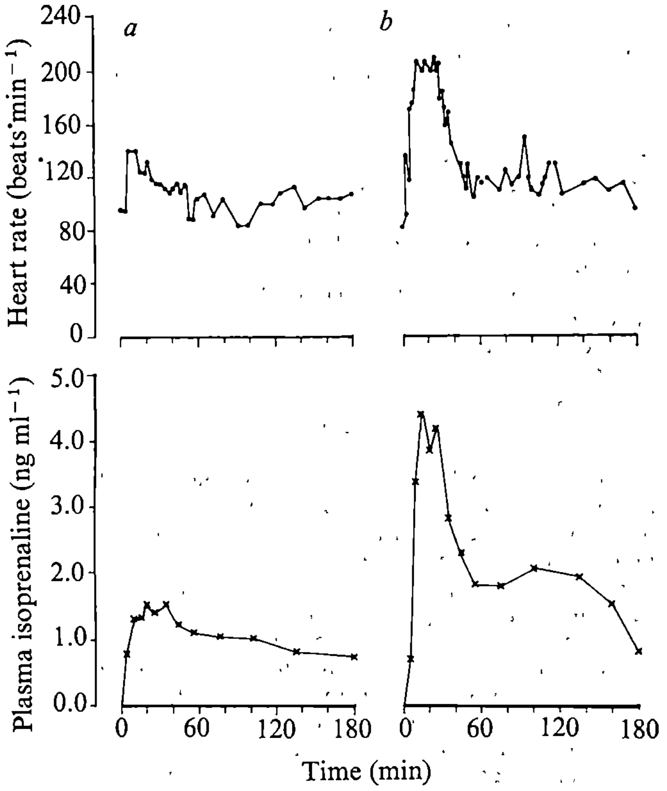

Fig. 1 Plasma free isoprenaline and heart rate responses in a conscious greyhound which received: *a*, isoprenaline (3.0 mg base) or *b*, isoprenaline (3.0 mg base) with salicylamide (600 mg) on separate days.

was greater in each animal (mean 4.67±1.38 ng ml^{-1}) as was the area under the plasma isoprenaline–time curve (mean 386.9±50.6 ng ml^{-1} min). The results of a typical experiment are shown in Fig. 1.

In each animal the heart rate response, expressed as the peak heart rate from baseline and as the area under the heart rate-time curve calculated by trapezoidal rule, was greater when isoprenaline was given with salicylamide than when it was given alone (Table 1). In three animals given L-cysteine with isoprenaline and salicylamide, heart rate responses were less than those for isoprenaline and salicylamide and were similar to the responses for isoprenaline alone.

To assess whether salicylamide has any direct effect on the responsiveness of the dog heart to isoprenaline, an intravenous infusion of isoprenaline sulphate, 0.019 μg (base) kg^{-1} min^{-1}, was given to a conscious dog which had received atropine, 40 μg kg^{-1}, intravenously. When a stable increase in heart rate had been achieved, 80 mg of salicylamide was given intravenously. The mean (±s.d.) heart rate was 134±14 beats min^{-1} for 12.5 min before salicylamide and 130±12 beats min^{-1} for 12.5 min after salicylamide. These results are not significantly different at the 5% level of probability.

Sulphate exists in an activated form in the gut[7] and under the influence of sulphokinases, which exist throughout the gut[8], it can react with many substances, especially those with phenolic groupings. Salicylamide is metabolised principally to sulphate and glucuronide conjugates, and Levy and Matsuzawa[6] showed in man that the fraction of the dose excreted as salicylamide sulphate decreased markedly as the oral dose of salicylamide was increased from 75 to 2,000 mg. They also showed that co-administration of L-cysteine, which is catabolised to inorganic sulphate, restored the conjugation of salicylamide even at high doses, indicating that the capacity was limited by the availability of sulphate. It seems likely that the explanation for the increased plasma isoprenaline and increased cardiac responses when isoprenaline and salicylamide were administered together lies in competition between the two compounds for available sulphate in gut mucosa, enabling more free isoprenaline to reach the systemic circulation. The reduced cardiac response when L-cysteine was administered with salicylamide and isoprenaline is consistent with this hypothesis since availability of sulphate would not in this case be rate limiting. Thus the systemic availability of oral isoprenaline which is normally limited by metabolism in the gut wall can be increased by co-administration of another drug which shares its route of metabolism. Moreover, we have found that the increased availability and pharmacological effects are of considerable magnitude.

Although isoprenaline is commonly administered by inhalation of an aerosol, most of an inhaled dose is known to be swallowed and absorbed through the gut[9]. We have no evidence of interaction between isoprenaline or similar drugs and salicylamide in clinical use, but events of this type may be difficult to identify. One reason may be self-medication, for salicylamide is a constituent of some proprietary mixtures obtainable without prescription. Indeed it is possible that ascorbic acid (vitamin C) which can reduce the conversion of salicylamide to salicylamide sulphate[10], and paracetamol[11] which can compete with salicylamide for sulphate may also reduce the sulphate conjugation of isoprenaline, and both these substances are readily available to the public. Thus interactions between sympathomimetics which form conjugates with sulphate and other drugs which are similarly metabolised, seem to be a realistic possibility and would be potentially dangerous.

It is interesting that no clear explanation has been found for the rise in asthma deaths associated with the use of aerosol bronchodilators[12]. The interaction we describe illustrates a mechanism whereby toxic amounts of sympathomimetic drugs, even administered by aerosol, may reach the systemic circulation. Viewed from a different aspect, competition between substances which are metabolised by conversion to sulphate conjugates might be used to enhance the availability and therapeutic usefulness of a drug which is normally inactivated by gut mucosa.

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Caffeine inhibits cell transformation by 4-nitroquinoline-1-oxide

SOMATIC mutation has been considered a likely initiating step in chemical carcinogenesis[1–3], chiefly because of the close correlation between the mutagenic and carcinogenic activity of various chemicals[4–6]. Study of cells from patients with disease predisposing to a high incidence of cancer, such as xeroderma pigmentosum and Fanconi's anaemia, has suggested that defective DNA repair is correlated with this predisposition to malignant transformation. When cells are transformed by 3-methylcholanthrene[7], 4-nitroquinoline-1-oxide (4NQO)[8], or X irradiation[9–11], division is necessary soon after treatment to fix the transformation: if treated cells are unable to grow the transformation is not fixed. These results could be explained if DNA damage induced by a carcinogen is converted into a stable and replicable structural change only by means of DNA replication before the intervention of error-free repair mechanisms. The model of mutagenesis[12–14] based on evidence obtained with micro-organisms suggests that errors introduced by an error-prone postreplication repair mechanism are the principal source of mutation. Furthermore, caffeine inhibits "postreplication repair" (presumably an error-prone mechanism) but not excision repair (presumably error-free) in rodent cells[15–19]. I now report that caffeine reduces the transformation frequency of mouse cells treated with 4NQO. This supports the somatic mutation theory of cell transformation.

Malignant transformation was assayed in A31-714 cells, a subclone isolated from BALB/3T3-A31 clone, and 4NQO which induces transformation in these cells after a short exposure[20]. Actively growing cells were treated with 4NQO for 30 min in suspension, washed and then plated into 60-mm plastic dishes containing 5 ml of Eagle's minimal essential medium supplemented with 10% newborn or foetal bovine serum. Plating efficiency was determined 10 d later, and transformation was scored 30 d after treatment as described previously[20]. Caffeine was dissolved in Dulbecco's PBS, filtered, and added to culture medium. Because of the need for cell proliferation for the fixation and expression of transformation[8], plating efficiency was determined initially for all experimental conditions, and then the number of cells plated was adjusted, so that the average number of divisions required to reach confluence for the surviving cells was nearly the same in all dishes.

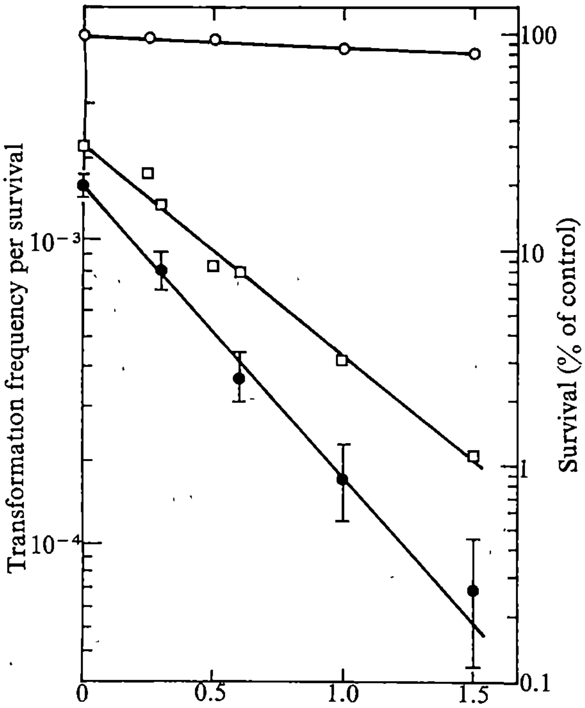

Fig. 1 Survival and transformation frequency in A31-714 mouse cells treated with 4NQO and subsequently incubated with various concentrations of caffeine. The graph shows surviving fraction of untreated (○), and of 4NQO-treated cells (□); transformation frequency (mean ± s.e.) of 4NQO-treated cells (●). Actively growing cells were treated with 4NQO (0.1 µg ml^{-1}) for 30 min in suspension, washed with Dulbecco's PBS, and plated at an inoculum such that about 1×10^4 cells per 5-cm dish survived for the transformation assay and 30–100 cells per dish survived for the assay of plating efficiency. Caffeine was present in the culture medium for the first 48 h after plating. The medium was then replaced with fresh, caffeine-free medium and was subsequently changed twice a week. Dishes were then fixed with methanol and stained with Giemsa either 10 or 30 d after plating and scored for plating efficiency and transformation frequency, respectively. The s.e. of the plating efficiency was always less than 3% of the corresponding mean value.

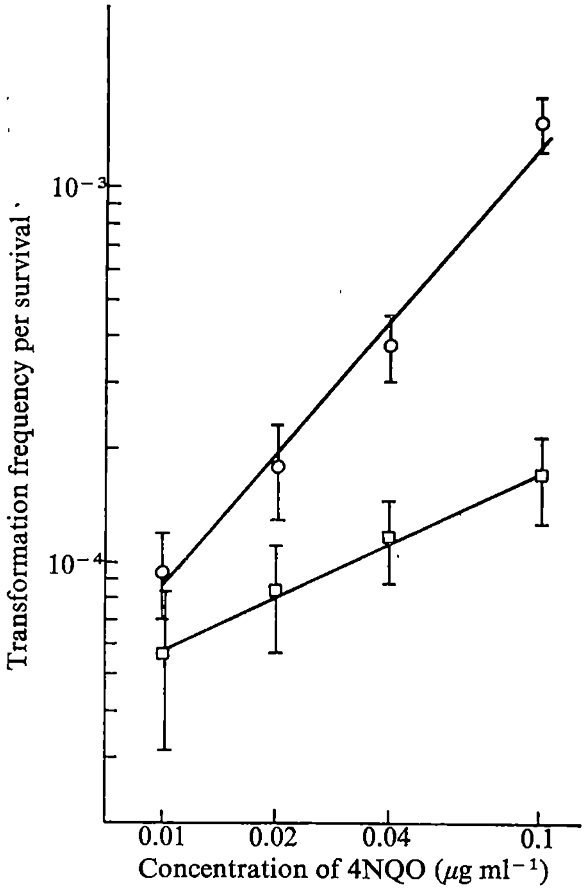

Fig. 2 Transformation frequency (mean ± s.e.) in A31-714 mouse cells treated with various concentrations of 4NQO in the presence (□) or absence (○) of 1 mM caffeine for 48 h after treatent were 4NQO. See legend to Fig. 1.

Figure 1 shows the dose–response curves for both transformation and survival of A31-714 cells treated with 4NQO (0.1 µg ml^{-1}) in the presence or absence of caffeine for the first 48 h after treatment. The upper two curves, which represent cell survival, show that caffeine reduced the survival of treated cells but not that of untreated cells. Transformation frequency per surviving cell, shown by the lower curve with the vertical bars, was also reduced by caffeine. Both survival and transformation frequency of treated cells were inversely proportional to the concentration of caffeine and almost parallel within the range of concentrations of caffeine tested. As Fig. 2 shows, the inhibitory effect of caffeine on transformation frequency was also dependent on the concentration of 4NQO.

The exposure of caffeine to cells before, or 48 h after treatment with 4NQO (0.1 µg ml^{-1} for 30 min) had no marked effect on survival and transformation frequency (Table 1). The presence of caffeine for the first 24 h after treatment with 4NQO caused much more reduction in survival and transformation frequency than in successive 24 h periods (Table 1). When the transformation frequency was calculated on the basis of the number of cells inoculated, the inhibitory effect of caffeine was even greater (not shown). In the conditions of my experiment, most cells which did not grow rapidly became detached from the substrate, so that 24 h after plating less than 10^5 cells per dish were attached to the substrate, and less than 2×10^4 cells per dish were attached after 48 h. As reported previously[8], these cell densities affect neither the fixation nor the expression of transformed state.

My results are apparently consistent with the finding that caffeine inhibits tumour induction by ultraviolet irradiation in mice *in vivo*[21]. The discrepancy between my results and those of Donovan and DiPaolo[22], in which caffeine appeared to enhance the chemically induced morphological transformation of hamster embryonic cells, may be due to differences between the transformation assays used, or to the length of time and the sequence of carcinogen and caffeine treatment In their experiments, cells were exposed to carcinogens for 48–49 h simultaneously with, or beginning 1 h before, addition of caffeine.

Table 1 Survival and transformation frequency of 4NQO-treated A31-714 cells in the presence of caffeine added at various times

Presence of 1 mM caffeine (h after 4NQO-treatment)	Plating efficiency 10 d after treatment (%)	Transformation frequency per survival* ($\times10^{-4}$)
	26	12.7±1.7
−24–0	23	13.9±1.7
0–48	2.4	1.5±0.4
0–96	1 9	1.8±0.6
48–96	16	12 5±2.3
0–24	4.1	3.0±0.8
24–48	11	6.5±1.1

*Mean±s.e.

In mammalian cells, the most marked effect of caffeine is its potentiation of cell killing and induction of chromosome aberrations by ultraviolet light and alkylating agents[23]. These are caused by low doses of caffeine, which by themselves do not have significant effects on cell survival, as my experiments have also shown. It has been suggested that this synergistic effect of caffeine is due to its inhibition of "postreplication repair", because all results reported so far show that caffene inhibits the elongation of newly synthesised daughter strand DNA after ultraviolet irradiation of rodent cells[15–19]. With the A31-714 cells used in this experiment, 1 mM caffeine does not inhibit 4NQO-induced unscheduled DNA synthesis[24]. I suggest that the reduction by caffeine of survival and transformation frequency of 4NQO-treated A31-714 cells is related to the observation that caffeine inhibits "postreplication repair" in mouse cells. Thus if "postreplication repair" is a viable error-prone repair mechanism, its inhibition would be expected to have the results reported here. This is compatible with the suggestions that (1) cell division soon after carcinogen treatment is required for the fixation of transformation[7–11], and (2) late G_1 cells show the highest frequency of transformation by radiomimetic alkylating agent[25].

My results seem to support the repair–error hypothesis[14,24,26] as a mechanism for chemical and physical carcinogenesis According to this hypothesis, carcinogen-induced DNA damage can be repaired by at least two repair mechanisms, one sensitive to caffeine and the other not. The former may be equivalent to the so-called "postreplication repair" which occurs chiefly during or soon after DNA replication and is presumed to be error prone. The latter, however, may be equivalent to excision repair which occurs throughout the cell cycle, and is presumed to be error free. The errors in the former repair system would then be the main source of mutations, some of which may lead to cell transformation.

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Local membrane current in *Limulus* photoreceptors

IT has been shown[1] that when part of a cell of the ventral photoreceptors of *Limulus* is illuminated, light adaptation is localised to the region of illumination. Since, in the vertebrate retina, there is a well known component of adaptation that is correlated with pigment concentration[2–4], local adaptation in *Limulus* may similarly be related to pigment bleaching in the illuminated region of the photoreceptor. This has been shown to be improbable, however, by experiments which indicate that adaptation in the photoreceptor is unrelated to the recovery of the visual pigment[5,6].

Another possibility is that local adaptation is related to some process that is initiated by the visual pigment. Voltage clamp studies on ventral photoreceptors[7] have shown that illumination initiates an inward flow of membrane current, brought about by an increase in the underlying conductance. If the inward current and the underlying conductance increase were localised to the region of illumination, then it might be possible that either, or both, of these processes are in some way responsible for local adaptation. We have therefore attempted to demonstrate directly the flow of local membrane current in the ventral photoreceptors of *Limulus*.

The ventral photoreceptors are located on the so-called lateral 'olfactory' nerve, which was removed from the animal, desheathed, and pinned to the bottom of a chamber with a transparent base. A photoreceptor was stimulated from below, using an optical bench so that a small movable spot of light (nominally 30 μm diameter in these experiments) could be positioned anywhere on these large photoreceptors (typically 50×150 μm in cross section[8]). Under visual control (×400 compound microscope), a portion of a single photoreceptor was gently drawn into a glass suction electrode. Then a KCl filled micropipette (resistance, 10–30Ω measured in the Ringer that bathed the preparation) was inserted into the cell. We were thus able to measure, simultaneously, the extracellular currents using the suction electrode, and the receptor transmembrane potential using the intracellular pipette. Figure 1*b* shows a

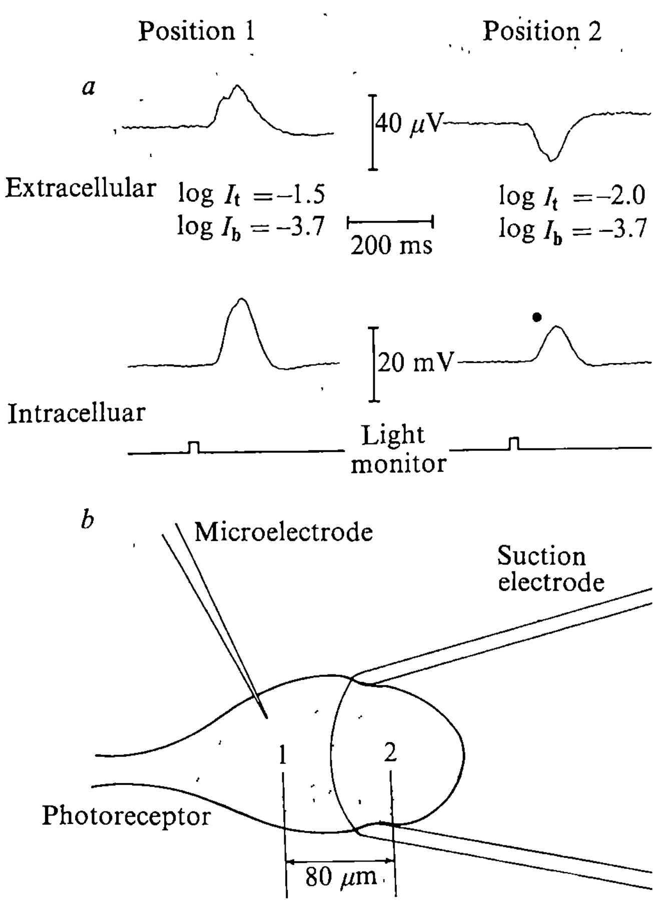


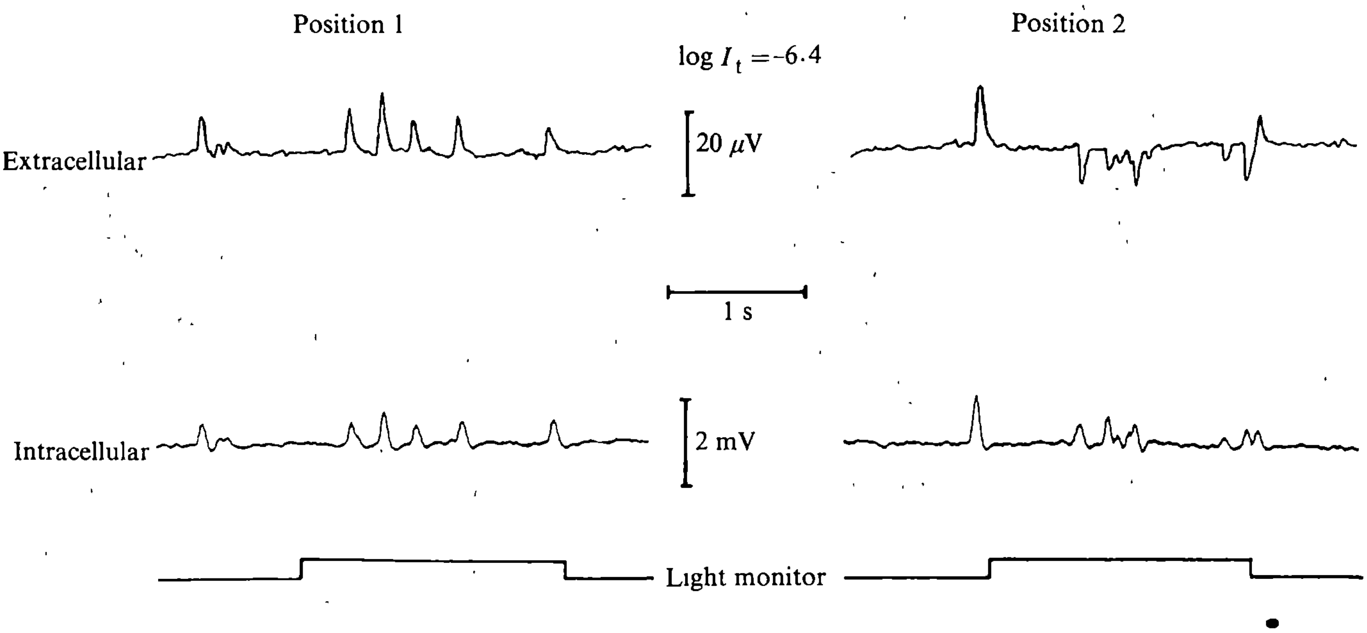
Fig. 1*a*, Local membrane currents produced by local illumination in a light-adapted photoreceptor. *b*, Schematised version of the recording situation, showing the two stimulus spots labelled 1 and 2. Light intensities (I) are given as $\log_{10} I/I_0$ where I_0 is the intensity of the unattenuated beam. I_t is the intensity of the test flash, and I_b is the intensity of the full-field steady background. The extracellular recording was made between a chloride-coated silver wire inside the suction electrode and a chloride-coated silver wire in the bath. An a.c. coupled amplifier with a 0.1-Hz low frequency cutoff was used for the extracellular recording.

schematic representation of the recording and stimulating situation. The test spot was located at either of two positions, designated 1 and 2. The aim was to stimulate two different regions of the photoreceptor, one inside, the other outside the suction electrode.

Figure 1 indicates that local illumination gives rise to a localised flow of inward membrane current. When the region inside the suction electrode was illuminated (position 2 Fig. 1), a flow of current from the bath into the suction electrode was measured. The path was completed when the current flowed into the illuminated region of the cell, inside the suction electrode, and out of the cell through the unilluminated regions, outside the suction electrode. Similarly, when the photoreceptor was stimulated at a region outside the suction electrode (position 1, Fig. 1*b*) we measured a flow of current from the suction electrode into the bath. As before, the path was completed when the current flowed into the illuminated region of the cell, this time in the region outside the suction electrode, and out of the cell through the unilluminated regions, this time located inside the suction electrode. In both cases the receptor current depolarised the cell, which was shown by the intracellular recordings of the receptor transmembrane potential. These results indicate that localised illumination of these photoreceptors leads to a flow of local membrane current. This finding is similar to the observation of local membrane currents in the outer segment of squid photoreceptors[9]. The observations on squid photoreceptors, however, did not indicate which changes might bring about the local membrane current. As mentioned previously, the voltage clamp technique has shown that illumination initiates a flow of inward membrane current in *Limulus* ventral photoreceptors, that is brought about by an increase in conductance. Our results therefore indicate that a localised conductance increase gives rise to the local inward membrane current, produced by the localised illumination.

When dark-adapted *Limulus* photoreceptors are stimulated with dim flashes, discrete waves of depolarisation (Fig. 2), sometimes referred to as 'quantum bumps', are observed. These 'quantum bumps' have been observed in a variety of arthropod photoreceptors[7,10–13], and in ventral photoreceptors they seem

Fig. 2 Local membrane currents associated with 'quantum bumps' in a dark-adapted photoreceptor. Positions 1 and 2 are the same as those shown in Fig 1*b*. The recording situation was the same as that shown in Fig. 1, but the data shown are from a different cell than in Fig. 1. I_t is the intensity of the test flash. Note what seems to be 'spontaneous quantum bumps' preceding the light stimuli. The above records show only one polarity of these 'spontaneous quantum bumps'. In other records, not presented here, we have also observed 'spontaneous quantum bumps' of opposite polarity. These 'quantum bumps' seem to be spontaneous but we cannot rule out the possibility that they are produced by stray light.



to be produced when a photon excites a single rhodopsin molecule[14]. It has been proposed[15] that the receptor potential is the summation of these quantum bumps. Figure 2 shows that the individual quantum bumps are associated with local membrane currents. The results presented in Fig. 2 indicate that when a single photon excites one rhodopsin molecule, this process initiates a localised conductance increase which, in turn, generates a flow of local membrane current. We suggest that either the localised light-induced conductance increase, or the local membrane current which it generates, or possibly both, are somehow involved in the local adaptation that follows local illumination.

These results can also be interpreted in conjunction with the Ca^{2+} hypothesis of Lisman and Brown[16]. They suggested that a light-induced increase in intracellular Ca^{2+} concentration (Ca_i^{2+}) is a factor controlling light adaptation in *Limulus* ventral photoreceptors. It would seem that following local illumination, the light induced conductance increase and/or the inward current that it initiates, leads to a local rise in Ca_i^{2+} and thereby to local adaptation. This interpretation is supported by the findings of Fein and Lisman[17], who showed that injection of calcium ions into ventral photoreceptors locally desensitised the photoreceptor.

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Intracellular Ca modulates sensitivity and time scale in *Limulus* ventral photoreceptors

IN a photoreceptor cell, light induces a change in membrane voltage (the 'receptor potential') The sensitivity of the photoreceptor (defined as the amplitude of the receptor response per unit of stimulus energy) depends on its history of illumination. After a cell has been stimulated by light, its sensitivity becomes reduced (that is, the cell becomes 'light adapted'). Fuortes and Hodgkin[1] reported that previous illuminations not only reduced the sensitivity of photoreceptors in *Limulus* lateral eye but also decreased the latency of the responses to subsequent stimuli. They found that the reduction in latency (referred to as a shortened 'time scale') was greater with brighter adapting lights, and that the shortening of the time scale was much smaller proportionally than was the reduction in sensitivity. Similar changes in time scale also have been found in *Limulus* ventral photoreceptors[2,3].

It has been proposed that a light-induced increase of intracellular free calcium concentration, Ca_i, normally modulates the sensitivity of *Limulus* ventral photoreceptors[4]. The evidence for this proposal is: (1) intracellular injections of calcium ions decrease the amplitude of the receptor potentials[4]; (2) intracellular injection of a calcium buffer tends to prevent light-induced changes in sensitivity[5]; and (3) a light-induced rise of Ca_i has been detected directly with the photoprotein aequorin[6]. Here we show that iontophoretic injection of Ca into *Limulus* ventral photoreceptors not only decreases the sensitivity but also shortens the time scale.

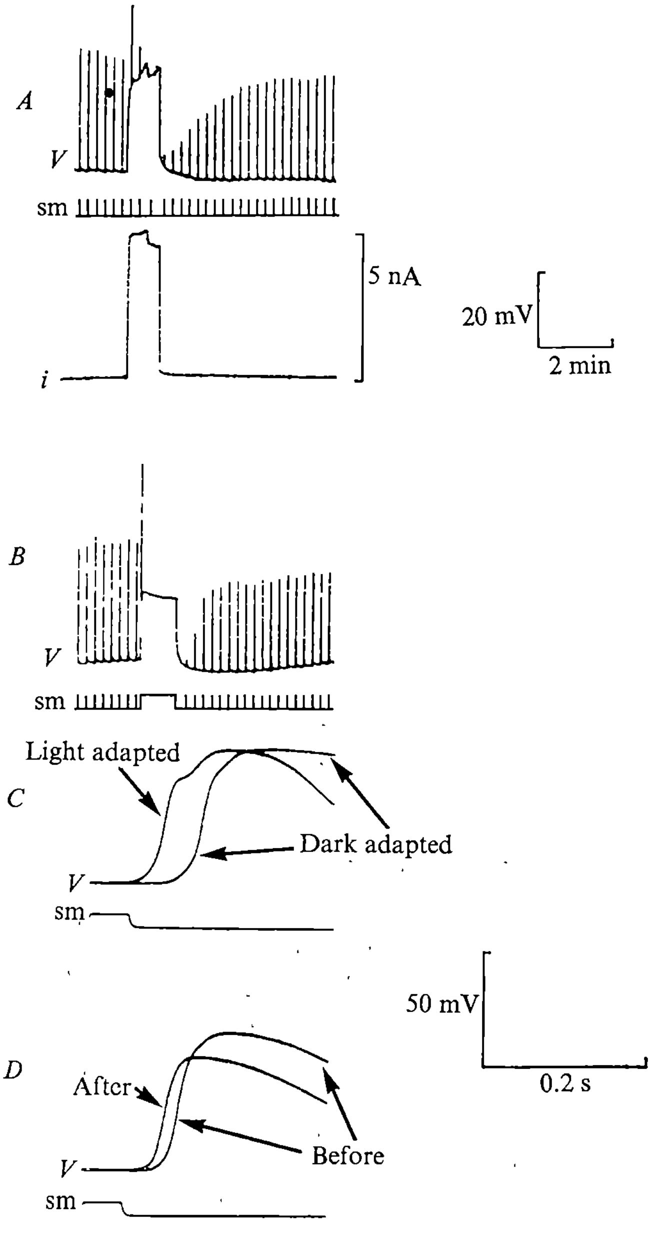
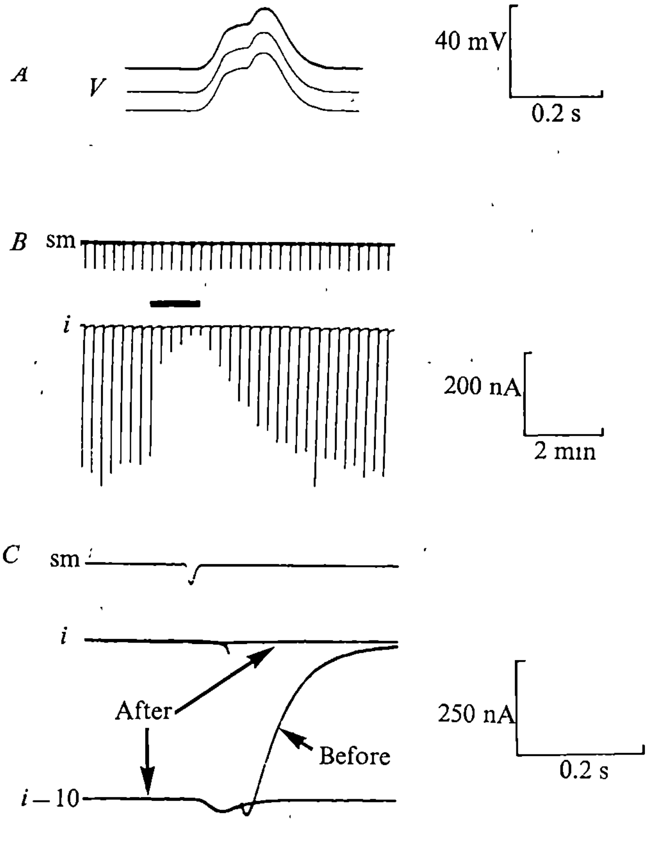


Fig. 1 The amplitude and latency of receptor potentials recorded from *Limulus* ventral photoreceptors, altered by bright lights or by intracellular injection of calcium. *V*, Voltage; sm, monitor of the time of occurrence of stimulus light. A micropipette filled with 3 M KCl was used to measure membrane voltage; a second micropipette filled with 0 09 M CaOH, 0.1 M EGTA and 0.1 M Tris base was used to pass Ca iontophoretically into the cell (refs 4 and 11). Unattenuated stimulus beam was 5×10^{-3} W cm^{-2} on the preparation. *A*, Responses to repetitive brief (20 ms) test flashes (stimulus beam attenuated by 3.9 neutral density) before and after the iontophoretic injection of Ca_i monitor of the current through the electrode containing Ca. *B*, Responses to repetitive brief (20 ms) test flashes (attenuation = 4.8 neutral density) before and after a prolonged adapting stimulus. After the adapting light, the responses to test flashes gradually recovered. Same cell as *A*. *C*, In another cell, the response to a flash (attenuation = 3 6 neutral density) before (dark adapted for 15 min) and 10 s after an unattenuated adapting stimulus. *D*, Same cell as *C*. The response to a flash (attenuation = 3.6 neutral density) before and 10 s after iontophoretic injection of Ca. The cell had been dark adapted for 15 min before each flash.

As described previously[4], receptor potentials elicited by brief repetitive flashes are attenuated during the iontophoretic injection of Ca, and recover gradually after the injection (Fig. 1*A*). This phenomenon qualitatively mimics the desensitisation of the cell by an adapting light and the subsequent recovery of sensitivity (Fig. 1*B*).

It has been inferred[4] that the attenuation of the receptor potential by intracellular injection of calcium ions is probably due to the attenuation of the light-induced conductance. To examine directly the effects of injection of calcium ions on the light-induced conductance, cells were voltage clamped during the injection. A cell was impaled with two electrodes filled with KCl, for the voltage clamp, and also impaled with a third electrode for injecting calcium ions iontophoretically. The voltage clamp supplied a current equal and opposite to the current passed out of the Ca-containing electrode; if the inside of the cell was equipotential, no net current would cross the cell membrane due to Ca iontophoresis. Before a cell was voltage clamped, the voltages (receptor potentials and resting voltage) recorded by all three electrodes were found to be nearly identical (Fig. 2*A*). The cell was then voltage clamped, and the currents induced by brief dim repetitive flashes were measured. During the iontophoretic injection of Ca the light-induced current became progressively smaller, and after the injection it progressively recovered (Fig. 2*B*). The intensity of the repetitive flashes was chosen to be within the linear portion of the relationship between flash intensity and peak light-induced current; therefore, the reduction in light-induced current was directly proportional to the reduction in the sensitivity of the cell. Thus, the direct intracellular injection of Ca caused a reduction of the sensitivity of the photoreceptor, in the absence of a change of membrane voltage. We have also found that intracellular iontophoretic injection of calcium ions does not noticeably change the reversal voltage of the current induced by a brief flash Thus, with membrane voltage held constant, the light-induced current is directly proportional to the light-induced conductance. We conclude that the finding illustrated in Fig. 2*B* directly demonstrates that an increase of intracellular free calcium modulates the light-induced change in membrane conductance which underlies the photoreceptor response. It has been similarly demonstrated that bright illumination reduces the conductance increase evoked by subsequent stimuli[7].

Fig. 2 A cell was impaled with two KCl-filled microelectrodes and a third electrode containing Ca solution. The unattenuated stimulus light was 5×10^{-3} W cm^{-2}. *A*, From the dark-adapted cell the three electrodes recorded identical receptor potentials to a brief flash (attenuation = 4.8 neutral density). *B*, Current (*i*) induced by repetitive 50-ms test flashes (attenuation = 4.2 neutral density; sm, stimulus monitor) before, during, and after iontophoretic injection of Ca. Duration of injection indicated by bar. Holding voltage equal to dark resting voltage. The injection caused no change in the membrane current measured in the dark (between flashes). *C*, Current (*i*) induced by test flashes (attenuation = 3.0 neutral density) before and after iontophoretic injection of Ca while cell was voltage clamped at dark, resting voltage. Response after injection is shown at two amplifications, differing by a factor of 10.



Using brief bright stimuli, a dramatic shortening of the time scale of the receptor potential was found after the photoreceptor was light adapted (Fig. 1*C*). To see if an increase of Ca$_i$ affected the time scale in addition to reducing the sensitivity, we measured membrane voltage during and after iontophoretic injection of calcium ions. Shortening of time scale also occurred after the intracellular iontophoretic injection of Ca (Fig. 1*D*). Moreover, the shortening of time scale did not depend on passing net current across the cell membrane during the iontophoretic injection; injection of Ca while the cell was voltage clamped shortened the time scale (Fig. 2*C*) and also decreased the sensitivity. Thus, increasing intracellular free calcium mimics two effects of previous illumination: a large reduction of sensitivity and a proportionally smaller shortening of the time scale. Injection of calcium ions does not, however, mimic the excitatory effect of light. Figure 2 shows that membrane current in the dark (measured at resting voltage) was not affected by the injection. This clearly shows that, in *Limulus*, a rise of Ca$_i$ is not the signal for excitation, as has been proposed for vertebrate photoreceptors[8,9].

In summary, we have found that in ventral photoreceptors, changing a single quantity, the free intracellular calcium, leads to both a shortening of time scale and a reduction of sensitivity. Increasing calcium concentration has been found to attenuate stimulus-induced changes of membrane conductance channels in other membranes (compare 'stabilisation' effects of Ca; ref. 10). Our findings that intracellular injection of Ca not only decreases the sensitivity but also shortens time scale of the light-induced current suggests that the action of Ca$_i$ is probably not a nonspecific effect on the conductance channels. These findings further support the hypothesis that a change of Ca$_i$ specifically participates in the physiology of light adaptation in *Limulus* ventral photoreceptors.

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Selective release of newly synthesised and newly captured GABA from synaptosomes by potassium depolarisation

NORADRENALINE and acetylcholine synthesised *de novo* are released in preference to the endogenous store following nerve depolarisation. Kopin *et al.*[1] showed that the specific activity of noradrenaline (^{14}C-tyrosine precursor) released from cat spleen during sympathetic nerve stimulation is greater than that of the tissue store; however, ^{3}H-noradrenaline which was just taken up by the nerve is released concomitantly with the endogenous store. In similar experiments, newly synthesised acetylcholine is selectively released from superior cervical ganglia[2], phrenic nerve[3], *Torpedo* electroplax[4], and brain cortical slices[5]. We used rat cortical synaptosomes (pinched off nerve endings) to study the temporal relationships of GABA biosynthesis, uptake and release.

Suspensions, enriched in synaptosomes, prepared by standard subcellular fractionation techniques, take up alleged neurotransmitter substances (for example, γ-aminobutyric acid (GABA), L-glutamate, L-aspartate) against a concentration gradient by specific sodium-dependent high affinity (low K_m) transport systems[6]. These high affinity transport systems are thought to be important in inactivating neurotransmitter action. Following electrical stimulation[7] or calcium-dependent potassium depolarisation[8,9], preparations enriched in synaptosomes specifically release neurophysiologically active amino acids (GABA, L-glutamate, L-asparatate). Using ^{14}C-L-glutamate as a precursor to measure GABA biosynthesis *de novo* and low concentrations of ^{3}H-GABA as a substrate for the high affinity uptake system, we find that newly synthesised GABA is released from synaptosomes concomitantly with the GABA taken up by the high affinity transport system. Moreover, both newly synthesised and captured GABA are released in preference to endogenous GABA stores.

GABA is a probable transmitter in the vertebrate central nervous system[10] and the established neurotransmitter at the crustacean neuromuscular junction[11]. Although usually considered an inhibitory neurotransmitter, we suggested that GABA may be the excitatory transmitter of the olfactory nerve in the gar fish and pike[12]. Glutamate decarboxylase catalyses the biosynthesis of GABA according to

$$\text{L-glutamate} \rightarrow \text{GABA} + CO_2$$

This enzyme, which requires pyridoxal phosphate[13], is strongly inhibited by carbonyl-trapping reagents such as hydroxylamine and hydrazine[14], and also by mercaptosuccinate (our unpublished observations).

Using male Sprague–Dawley rats (200–250 g), fractions enriched in synaptosomes were isolated from crude mitochondrial suspensions on discontinuous Ficoll gradients[15] and stored at 0 °C in calcium-free Ringer[9] containing 20 mM morpholinopropane sulphonic acid (pH 7.4). All incubations were completed within 7 h after the animals were killed. To remove ^{14}C-GABA and other impurities, commercial ^{14}C-(U)-L-glutamate (237 Ci mol^{-1}) was subjected to paper electrophoresis in 8% acetic acid–2% formic acid. Commercial ^{3}H-GABA (36.7 Ci mmol^{-1}) was free from impurities using this system. Preparations enriched in synaptosomes always contain adventitious microsomes and myelin. Control studies which we report show that the conclusions are unaltered by this contamination.

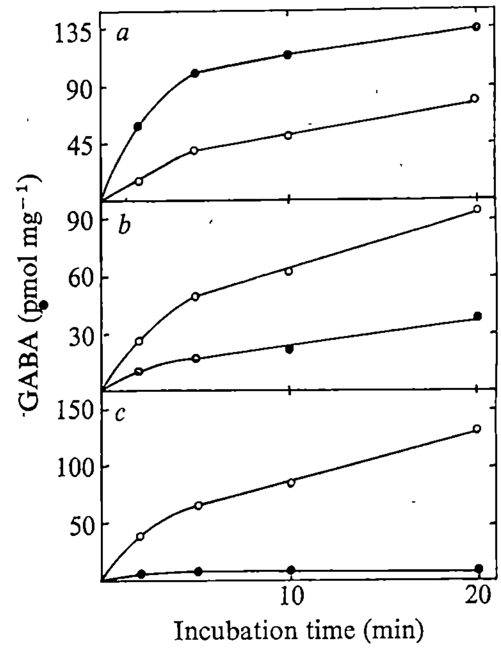

Fig. 1 Variation of newly synthesised and newly captured GABA in synaptosomes by changing exogenous substrate concentrations. A 90-µl aliquot of synaptosomal suspension (50–60 µg protein) was incubated at 25 °C for the time indicated with 10 µl of solution containing ^{14}C-L-glutamate and ^{3}H-GABA in Ringer's solution to give the following final respective concentrations: *a*, 30 µM–0.6 µM; *b*, 30 µM–0.3 µM; *c*, 30 µM–0.05 µM. Synaptosomes were collected on Millipore filters (0.45-µM pore). The filters were placed on Whatman 3MM paper and electrophoresed in 8% acetic acid–2% formic acid at 30 V cm^{-1}. After drying, carrier GABA was visualised with ninhydrin and quantitatively analysed for newly synthesised (^{14}C) (○) and newly captured (^{3}H) (●) GABA in Tritosol[18] using the channel ratios method[19]. Results are expressed per mg synaptosomal protein. In the conditions used, tritiated GABA was 17,000 c.p.m. pmol^{-1} and ^{14}C-GABA (newly synthesised) was 94 c.p.m. pmol^{-1}. Similar results were obtained in 2–4 independent experiments.

After incubating synaptosome fractions with ^{14}C-L-glutamate (30 µM) and ^{3}H-GABA (0.6 µM) at 25 °C, the rate of accumulation of both radiolabels into synaptosomes isolated by Millipore filtration is linear for 4 min and gradually flattens out between 20 and 30 min. To assess the extent of metabolism of these substances, including the conversion of L-glutamate to GABA, the synaptosomal preparation was collected by centrifugation (6,000g, 5 min, 4 °C) after incubation for 30 min with each labelled precursor. Subsequently, a water extract of the synaptosomes was prepared, applied directly on to paper, and electrophoresed. The position of the label was determined with a radiochromatogram scanner and that of the amino acids was determined by the ninhydrin reaction. About 80–90% of the radiolabel comigrated with the precursor. Moreover, L-glutamate (3%) was converted into a substance which comigrated with GABA during paper electrophoresis using 5.5% acetic acid–pyridine (pH 4.2) or the acetic acid–formic acid system. Incubation of the synaptosomes with hydroxylamine (1 mM) or mercaptosuccinate (1 mM) inhibits GABA bioformation from ^{14}C-L-glutamate by 90% and 25%, respectively, as determined after resolving sequestered GABA and L-glutamate by paper electrophoresis. In addition the ratio of newly synthesised GABA (^{14}C-L-glutamate precursor) and newly captured GABA (^{3}H-GABA precursor) varies with the concentration of precursor used in the synaptosomal incubation medium (Fig. 1*a*–*c*). Decreasing the concentration of ^{3}H-GABA in the medium, for example, decreases its uptake into synaptosomes with an attendant increase in the ratio of ^{14}C-GABA (newly synthesised) to ^{3}H-GABA (newly captured).

After documenting the biosynthesis of GABA from

^{14}C-L-glutamate and the uptake of ^{3}H-GABA into the synaptosome preparation, the effect of potassium depolarisation on the release of newly synthesised and captured GABA was studied. Synaptosomes containing ^{14}C- and ^{3}H-GABA release the candidate neurotransmitter on treatment with 56 mM potassium–1 mM calcium (Fig. 2*a–c*) The ratio of newly synthesised to newly captured GABA (labelling ratio) released closely parallels that found intrasynaptosomally before (Fig. 2*a–c*) and after (data not shown) potassium depolarisation In the data shown (Fig. 2*a–c*), the labelling ratios of intrasynaptosomal and released GABA were (*a*) 0.58, (*b*) 2.49; (*c*) 22.8 (±8%). This shows that the GABA released (up to 70%) by potassium depolarisation consists of both newly captured and synthesised substance Although the intrasynaptosomal ratio of these two forms of GABA can be controlled by varying substrate concentrations, neither form is selectively released. These results reflect net release since the magnitude of re-uptake of labelled GABA during depolarisation cannot be estimated.

Next, the specific radioactivity of stored and released GABA was measured. After depolarisation, the material released initially contains newly synthesised (^{14}C) and captured GABA (^{3}H) of higher specific activity than that found in the synaptosomes before potassium–calcium treatment (Table 1). With longer incubations, the specific activity of each label decreases to a value similar to that found in the initial preparation. These results support the hypothesis that newly synthesised and captured GABA are released preferentially when compared with the endogenous GABA store

Since myelin and microsomes sequester GABA[9], experiments were carried out to test the effect of potassium depolarisation on GABA release from these contaminants. Experiments performed with synaptosome preparations demonstrate that part of the sequestered radiolabel sediments with the myelin–microsomal fraction on sucrose density gradients, whereas the major portion comigrates with the synaptosomal fraction[16] Depolarisation of the preparation enriched in synaptosomes substantially reduces label comigrating with the synaptosomal fractions, but fails to release label from the myelin–microsomal fractions. In a control experiment using a myelin–microsomal preparation, depolarisation also fails to release label. These results are consistent with the observations of Levy *et al*, who demonstrated that myelin and microsomal preparations fail to release GABA by potassium treatment[9] Our experiments demonstrate that release of labelled GABA from synaptosome preparations by potassium depolorisation is not adventitious, and confirms the contention that simulus-induced release is synaptosome specific

Table 1 Release of endogenous, newly synthesised and newly captured GABA by depolarisation

Time (s)	Total GABA released (nmol mg^{-1})	Synthesised (pmol per nmol total GABA)	Captured (pmol per nmol total GABA)
2	< 0.25	> 40	> 26
20	0.74	20.1	15.7
80	1.48	14.4	11.8
150	1.78	11.8	9.5

A synaptosome preparation (870 µg protein) was incubated in a final volume of 300 µl of Ringer containing 30 µM ^{14}C-L-glutamate and 0.6 µM ^{3}H-GABA for 20 min at 25 °C. Synaptosomes were collected, washed and depolarised for the time indicated with K^+-Ca^{2+} as described in Fig. 2. Fractions containing released GABA were lyophilised and redissolved in 250 µl of 10 mM acetic acid. Total GABA was measured by the enzymatic fluorimetric procedure of Kravitz and Potter[20]. Newly synthesised (^{14}C) and newly captured (^{3}H) GABA were determined as described in Fig. 2. Intrasynaptosomal levels of GABA (before depolarisation) were: total GABA—8.7 nmol mg^{-1} protein; newly synthesised—12.3 pmol per nmol total GABA; newly captured—8.3 pmol per nmol total GABA. All determinations were performed in triplicate Average values (range±9%) are given.

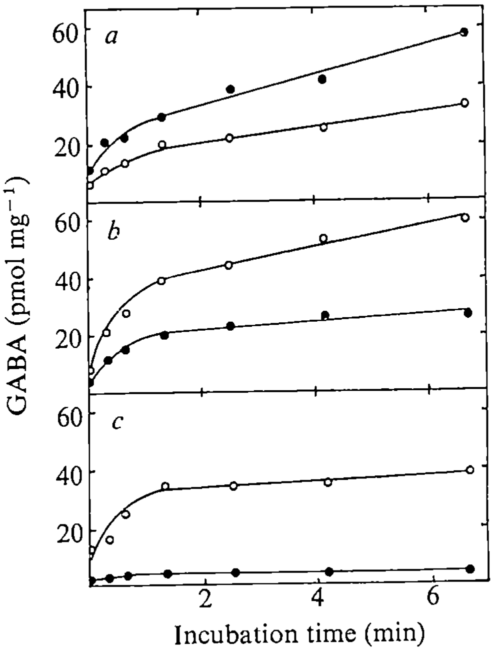

Fig. 2 Release of newly synthesised and newly captured GABA from synaptosomes. Synaptosomes were labelled with ^{14}C- and ^{3}H-precursor as described in Fig. 1*a–c* for 20 min, using 120–130 µg of synaptosomal protein in a final incubation volume of 250 µl. Synaptosomes were collected by centrifugation at 4,500*g* for 3 min and washed twice with 2.5 ml of cold Ringer to remove extrasynaptosomal isotope. The pellet was resuspended in 250 µl of Ringer (on ice); then 250 µl of Ringer containing 112 mM potassium chloride and 2 mM calcium chloride was added rapidly. After incubation at 25 °C for the time indicated, the components released into the medium were resolved from synaptosomes by Millipore filtration. The filtrates were lyophilised and redissolved in 100 mM acetic acid containing carrier GABA. Aliquots were electrophoresed on Whatman 3MM paper for 90 min in 5.5% acetic acid–pyridine, *p*H 4.2 at 15 V cm^{-1}. After drying, GABA was quantitatively analysed as described in Fig. 1. In the conditions used, the following concentrations (pmol mg^{-1}) of newly synthesised and newly captured GABA (respectively) were present before potassium depolarisation (see Fig. 1*a–c*): *a*, 80–137; *b*, 97–39; *c*, 130–5.7. ○, Newly synthesised, ●, newly captured. Similar results were obtained in 2–4 independent experiments.

A 30-µM concentration of L-glutamate is an effective precursor of GABA in the synaptosome preparation. The K_m of the glutamate high affinity uptake system, the process presumed to inactivate the action of this excitatory neurotransmitter candidate, is about 20 µM (ref 17). Whether glutamate transport into neurones using GABA as the neurotransmitter is mediated by a similar high affinity transport system remains to be clarified. Newly captured and newly synthesised GABA are released in ratios similar to those found in synaptosomes before depolarisation. Release of GABA is thus unlike that of noradrenaline in the peripheral sympathetic nervous system (splenic nerve) of cat, where the newly synthesised transmitter is released in preference to that which is newly captured. Based on our experiments with rat cortical synaptosomes, GABA reuptake is not only important for terminating transmitter action, but seems to serve as a rather direct source for release of GABA in subsequent nerve volleys.

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Purified hormones from the crustacean eyestalk and their physiological specificity

THE amino acid compositions of two peptide hormones, both acting on the pigmentary-effector system of crustaceans, have been reported[1]. One of these, erythrophore concentrating hormone (ECH) has a major function in the rapid physiological colour changes of crustaceans, by concentrating pigment granules of the erythrophores, and is the first crustacean eyestalk hormone described[2]. The second eyestalk hormone, discovered not long afterwards[3], moves the distal (DRP) and reflecting retinal pigments of dark-adapted *Palaemonetes* and *Palaemon* into the light-adapted position, but purified hormone has so far been tested on only the DRP. A third well characterised hormone, hyperglycaemic hormone (HGH), increases glucose concentration of the blood, but such hyperglycaemic responses may vary in cross tests among crustacean species[4–6]. The amino acid composition of HGH has been determined. A summary of six analyses of five different preparations of HGH from eyestalks of *Cancer magister* is shown in Table 1; specific analyses for tryptophan have not been made. Details of the separation and purification will be reported elsewhere.

The diversity of physiological responses produced by hormones from the crustacean eyestalk[7–9] raises the question of the number of different hormones involved, that is, is each type of response produced by a single hormone, or may one hormone be responsible for more than one effect?

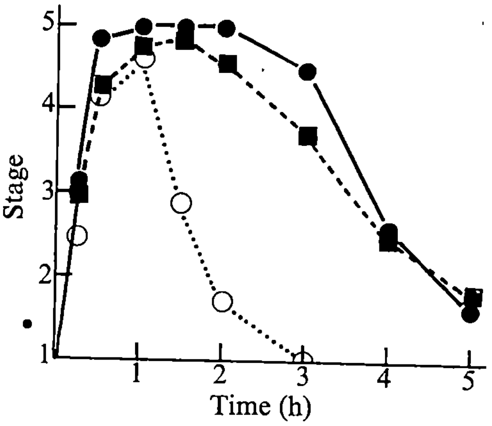

Fig. 1 Effects of synthetic light-adapting DRPH on chromatophores of *U. pugilator*. ●, Melanophores (10 animals); ■, erythrophores (10 animals); ○, leukophores (20 animals). Both eyestalks were removed from male crabs, average wet weight, 3.8 g (range 3.5–4.2 g) 18 h before testing to ensure that, at injection, melanophores and erythrophores were in stage 1. Normal males, averaging 1.6 g wet weight (range 1.4–2.1 g), were adapted to illuminated black backgrounds in individual fingerbowls so that the leukophore pigment was fully concentrated; after injection they were kept in the black containers except during the short intervals for observing the leukophore stage. The large individuals were injected with 0.2 μg and the small ones 0.1 μg of synthetic hormone (105 μg dissolved in 1.0 ml Pantin's saline[15] containing 1 μl of thiodiethanol). The hormone was stored at −20 °C between injections of groups of crabs. Chromatophore stages were read at ×36–×54 magnification.

The ideal approach is to test the specificity of response to pure hormone.

ECH has been tested for its physiological specificity on three crustacean species. Doses of 10^{-8} g of synthetic ECH concentrated the erythrophore pigments in *Uca pugilator* and in *Cambarellus shufeldti*, but were without effect on leukophores and melanophores of these species[10]. On the other hand, synthetic ECH in doses of 5×10^{-11} g, as well as hormone purified from eyestalks of *Pandalus borealis*, concentrated pigment both in leukophores and erythrophores of *Palaemon adspersus*[11] without eyestalks. The maximum leukophore response was small, an average stage 2.5 (on the Hogben and Slome[12] scale of 1–5) being reached 6 min after injection, with pigment fully dispersed again after the next 25 min. Although control injections are not described and leukophore responses to a range of doses of synthetic ECH are not reported, the results indicate differences in specificity of response to this hormone among crustacean species.

Table 1 Amino acid composition of hyperglycaemic hormone (HGH) from eyestalks of *C. magister*

Amino acid	Residues in samples 140–1	142–3	144–5	146–7C	146–7D	148–9A	Average number of residues	Range used	Estimated round number of residues
Lysine	2.5	4.0‡	2.2	1.9	2.0	2.2	2.16	1.9–2.5	2
Histidine	1.0	1.6	0.9	0.8	1.3	1.2	1.13	0.9–1.6	1
Arginine	2.2‡	3.5	3.5	3.3	3.0	3.2	3.30	3.0–3.5	3–4
Aspartic acid	7.7	6.6‡	8.0	8.2	7.6	7.8	7.86	7.6–8.2	8
Threonine	2.9	2.3	2.2	2.5	2.7	2.2	2.47	2.2–2.9	2–3
Serine	6.8‡	6.8‡	5.1	4.5	4.6	4.3	4.62	4.3–5.1	4–5
Glutamic acid	7.4	7.5	7.4	6.9	6.9	6.8	7.15	6.8–7.5	7
Proline	2.2	2.0	1.3‡	2.0	2.7	2.3	2.24	2.0–2.7	2
Glycine	10.0‡	6.4	4.8	5.6	5.5	4.4	5.34	4.4–6.4	5–6
Alanine	4.8	4.0	4.3	4.3	4.6	4.3	4.38	4.0–4.8	4–5
Half cystine*	0.6‡	0.8‡	1.4	1.1	1.1	3.8‡	1.20	1.1–1.4	1–2
Valine†	3.0‡	2.9‡	3.9	3.8	3.9	3.9	3.87	3.8–3.9	4
Methionine*	0.6‡	0.8‡	0.8‡	1.8	1.8	1.4	1.67	1.4–1.8	2 or more
Isoleucine*	1.0‡	1.3	1.5	1.4	1.6	1.6	1.48	1.3–1.6	2
Leucine	3.7‡	3.6‡	5.5	5.4	4.5	5.4	5.20	4.5–5.5	5
Tyrosine	3.6	3.0	4.0	3.8	3.7	4.2	3.71	3.0–4.2	4
Phenylalanine	2.0	2.0	2.2	2.2	2.3	2.3	2.17	2.0–2.3	2

*Cysteine, methionine and to some extent isoleucine are always low; also tryptophan if present.
†Valine not increased by 70-h hydrolysis time.
‡Values not used in averaging number of residues.

During purification studies on light-adapting DRPH[13,14], successive chromatography of eyestalk extract on columns of Sephadex G-25, DEAE–cellulose, and CM–cellulose revealed molecular polymorphism of the hormone. Each molecular version, in addition to light-adapting DRP, also dispersed pigments in melanophores, erythrophores and leukophores of *U. pugilator*. Such persistent association of physiological responses by the four pigmentary effectors indicated the likelihood of molecular identity between light-adapting DRPH and dispersers of chromatophoral pigments.

This possibility was tested with synthetic light-adapting DRPH on *Uca* (Fig 1). Pigment became dispersed in the three types of chromatophores, the maximum response occurring 30–60 min after hormone injection. Leukophores seem to be less sensitive to the hormone than melanophores and erythrophores. Doses of 2 ng produced an average stage 3.8 for melanophores in 6 eyestalkless *Uca* by 30 min after injection (not shown); the melanophores had returned to stage 1 after 3 h. Control injections of saline–thiodiethanol were without effect on the three kinds of chromatophores; five control animals were used for each chromatophore type.

The results show that light-adapting DRPH is also a disperser of chromatophoral pigments in *Uca*, and that a single hormone may elicit more than one physiological response. The role of purified HGH in the reported activation of phosphorylase and inhibition of uridine-diphosphate-glucose glycogen transglucosylase by eyestalk hormone[16,18] will be investigated.

Professor Rainer Keller, Drs Klaus-Dieter Spindler and Margaret Spindler-Barth were coworkers in the purification of HGH from *C. magister;* amino acid analyses of this HGH were done by Dr B. Brimhall (University of Oregon Medical School). Dr Per Fernlund provided a sample of synthetic light-adapting DRPH. Grants from the National Institutes of Health and from the National Science Foundation supported this research.

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Immunological cross reactivity between testicular androgen-binding protein and serum testosterone-binding globulin

WE have reported the presence of a testicular androgen-binding protein (ABP) which is secreted into the testicular fluid of several mammalian species[1]. ABP is produced by the Sertoli cells and is specifically stimulated by follicle stimulating hormone (FSH)[1–7] It is found in particularly high concentrations (3×10^{-7} M) in the efferent duct fluid of rabbits.

Rabbit serum also contains an androgen-binding protein (TeBG) with properties very similar to ABP[8–11]. In rabbits, ABP and TeBG are indistinguishable by size, stability and steroid-binding properties[12] (Table 1). To compare ABP and TeBG further, we have used antisera raised against purified ABP to investigate whether or not ABP and TeBG show immunological cross reactivity.

ABP was purified from 3,000 epididymides of adult rabbits by ammonium sulphate precipitation, ion exchange chromatography on DEAE–cellulose, gel filtration on Sephadex G-200, hydroxylapatite chromatography and preparative polyacrylamide gel electrophoresis[13]. In the purified preparation there was a thousandfold increase in specific ABP-binding activity compared with that of the 105,000*g* supernatant Purified (200 μg) or partially purified ABP (12 mg) was injected into young female guinea pigs after emulsification in 0.5 ml of Freund's complete adjuvant The animals were bled after 6 weeks and those showing an ABP titre were given booster injections to increase the titre. Antisera were checked for precipitin lines by double diffusion tests in agar[14]; and by inhibition of ABP-binding activity as measured by steady-state polyacrylamide gel electrophoresis[15].

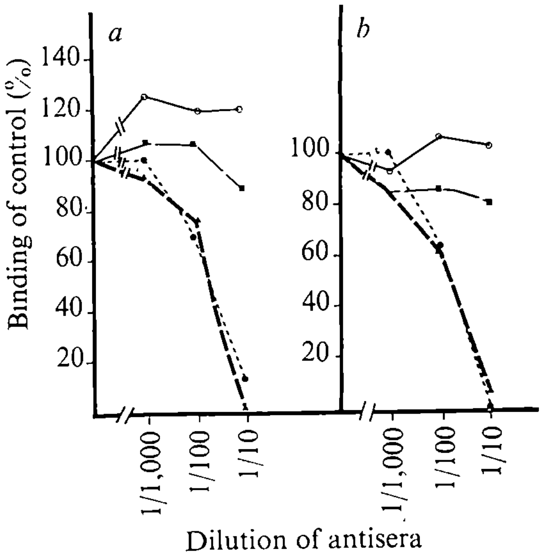

Fig. 1 Inhibition of ^{3}H-DHT binding to ABP (*a*) and TeBG (*b*) by different dilutions of anti-ABP antisera. Four guinea pigs were injected with partially purified rabbit ABP in Freund's complete adjuvant, and bled after 6 weeks. Various dilutions of antiserum (1/10, 1/100, 1/1,000) were added to an equal volume of partially purified ABP or immature rabbit serum (diluted 1:50, v/v). Normal guinea pig serum in the same dilution served as controls. After incubation for 48 h at 2–4 °C the samples were centrifuged at 10,000*g* for 15 min and binding of ^{3}H-DHT to ABP and TeBG was analysed by steady-state polyacrylamide gel electrophoresis[15]. There was no visible precipitation at any dilution of antisera ○, AS1; ●, AS2; ■, AS3; ▲, AS4.

As Fig. 1*a* shows, antisera from animals 2 and 4 caused 100% inhibition of ABP-binding activity at a dilution of 1 : 10. Some inhibition was also observed at a dilution of 1 : 100 but none at 1 : 1,000. Antisera from animals 1 and 3 showed little or no inhibition of steroid-binding activity, although they had activities against other antigens in the partially purified ABP preparation, as demonstrated by double diffusion tests in agar. Figure 1*b* shows that antisera raised in animals 2 and 4 also inhibited binding of ^{3}H-DHT to TeBG, and inhibition at different dilutions of antisera was identical to that of the inhibition of ABP-binding activity The finding that all four antisera showed precipitin lines in double diffusion tests in agar, and only two had a detectable titre against ABP and TeBG suggested that the inhibition was caused by specific antibody to ABP and TeBG and not by an artefact due to coprecipitation with contaminating antigens.

Table 1 Physicochemical properties of rabbit ABP and TeBG

	ABP	TeBG
$S_{20,w}$	4.7S (12) 4.5S (13)	4.4S (12)
Stokes radius (r_s)	43 Å (12)	44 Å (12)
Molecular weight ($S_{20,w}$, r_s)	74,000 (12)	76,000 (12)
Molecular radius (PAGE)	2.74 nm (12)	2.74 nm (12)
f/*fo* (molecular weight, r_s)	1.61 (12)	1.55 (12)
Molecular weight (PAGE)	75,000 (12)	75,000 (12)
Molecular weight (SDS–PAGE)	68,000 (13)	
Molecular weight (equilibrium ultracentrifugation)	65,500 (13)	
*p*I	4.7 (12, 13)	5.4 (12)
Ka^{DHT} at 0 °C	1.6×10^9 M^{-1} (12)	1.9×10^9 M^{-1} (12)
Steroid specificity	DHT > adiol > testosterone > progesterone > androstenedione > cortisol (12)	
Stability		
Binding destroyed by heat	> 50 °C (12)	> 50 °C (12)
Dissociation rate DHT–ABP complex ($t_{1/2}$ at 0 °C)	6 min*	5.2 min (11)

(v = 0.70 cm^3 g^{-1}). PAGE, polyacrylamide gel electrophoresis; SDS–PAGE, PAGE in 0.1% sodium dodecyl sulphate; *p*I, isoelectric focusing; *f*/*fo*, frictional ratio. DHT, dihydrotestosterone; adiol, 5α-androstane–3α,17β-diol. References are given in parentheses.
*Our unpublished results.

One explanation for the apparent immunological similarity between ABP and TeBG could be that TeBG is one of the proteins contaminating the ABP preparations used for immunisation, and that antibodies to both ABP and TeBG had been developed. To examine this possibility, more guinea pigs were immunised and antisera from different animals and bleedings were tested for inhibition of steroid binding to ABP and TeBG. As Fig. 2 shows, all antisera that inhibited steroid binding to ABP also inhibited binding to TeBG A plot of the binding to ABP against the binding to TeBG gave a positive correlation of 0.985. This high correlation between inhibition of steroid binding to ABP and TeBG strongly indicates that binding to both proteins is inhibited by the same antibody, and not by the products of different immune responses.

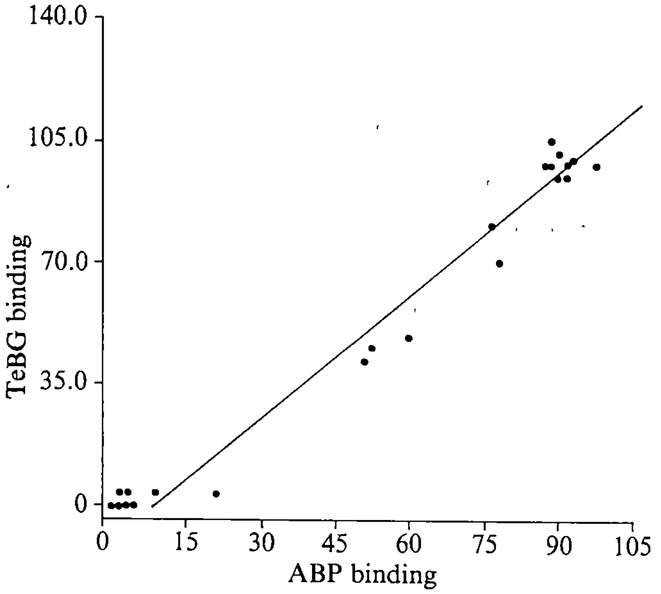

Fig. 2 Effect of 22 different antisera on ^{3}H-DHT binding to ABP (abcissa) and TeBG (ordinate). Antisera diluted 1:50 (v/v) were added to an equal volume of diluted rete testis fluid (1:20, v/v) or immature rabbit serum (dilution 1:20, v/v). Normal guinea pig serum served as control. After incubation for 48 h at 2–4 °C, the samples were centrifuged at 10,000*g* for 15 min and binding of ^{3}H-DHT to ABP and TeBG measured by steady-state polyacrylamide gel electrophoresis[15]. No visible precipitation was seen with any of the antisera used. Nine antisera caused no inhibition of ABP and TeBG binding Six antisera inhibited completely binding both ABP and TeBG. Seven antisera caused partial inhibition of both ABP and TeBG. Correlation coefficient for regression line = +0.985.

Thus, the present study shows that ABP and TeBG, in addition to having very similar physicochemical properties also show similar if not identical antigenicity ABP is produced in the Sertoli cells and secreted into testicular fluid while TeBG is present in the systemic circulation but of unknown origin. It is not likely that ABP originates in serum since proteins of this size cannot penetrate the blood–testis barrier[16]. ABP is also found in high concentrations in the rat, which lacks serum TeBG. Furthermore, Sertoli cells in culture release ABP into the medium and this process is stimulated by FSH[17,18]. It is also unlikely that TeBG originates in the testis, since high concentrations of TeBG are found in orchiectomised males as well as normal females[8–11]. Furthermore, ABP production is stimulated by FSH and androgens, while TeBG concentrations in serum are depressed by androgens and increased by oestrogens. Thus, ABP and TeBG might well be the same protein but produced at two different sites in the body and regulated by different hormonal mechanisms. Recent studies indicate possible differences in the carbohydrate portions of the ABP and TeBG molecules. TeBG is firmly bound by concanavalin A, while there is little or no binding of ABP (S C.W. *et al.*, unpublished) These studies suggest minor differences in the terminal sugars of the two proteins; and chemical analysis of purified preparations will be required to establish these differences further.

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DNA replication and accelerated chain growth in oestrogen-stimulated uterine cells

OESTROGEN has been shown to reduce S phase duration in uterine epithelial cells, although no agreement has been reached either on the exact values of cell cycle parameters, or even on the relative magnitude of the hormone-induced modifications[1-6]. It has also been shown that eukaryotic DNA molecules consist of replicative units arranged end-to-end, from the middle of which DNA synthesis proceeds bidirectionally at divergent fork growing points until fusion occurs with adjacent sections. The chain growth rate as well as the mean distance between initiation points were found to be specific for each cell type[7-13]. From this it can be inferred that oestrogen-dependent shortening of S phase in the uterine epithelium is due to speeding-up of replication along pre-existing units, or to multiplication of simultaneously operating replicative sections, or to both these mechanisms. The results that we have obtained from autoradiography of DNA fibres confirm that oestrogen increases the rate of DNA fork replication.

The microscopic measurements of grain tracks corresponding to replicated sections of DNA double helices show that with both ^{3}H-TdR pulse durations uterine epithelial DNA from oestradiol-treated mice contains labelled segments which are 50% longer than in controls (Figs 1 and 2). On the other hand, distances between centres of adjacent grain tracks do not seem to vary. Only in the experimental group given a 60-min labelling pulse were the values apparently somewhat higher. Although the comparison between experimental and control data is meaningful in terms of modifications of replication rate and density of replicative units, the calculation of true fork growth rate and initiation intervals from our data presents some difficulties which have already been partly discussed[12,13]. The histograms of track lengths are, in fact, an indeterminate mixture (see Fig. 3) of: (1) short sections arising from unidirectional replication of half-replicative units in which DNA synthesis was already under way at the start of pulse labelling; (2) labelled sections of short to medium length corresponding to bidirectional chain growth in units initiated at different times in the pulse periods; (3) average to long tracks due to fusion of adjacent replicons.

Frequencies of short tracks are probably overestimated since such segments are more often scored in the numerous short internal autoradiograms available for measurement. Being more prone to be broken or blurred by superposition with other fibres, suitable long tandem arrays are less often found. Consequently, fork replication rate, derived from halving the modal length of labelled segments is bound to be too low. For similar reasons centre–centre distances do not represent the true value of intervals between initiation points. Measurements involving fused sections tend to overestimate the interval length whereas those performed between partly labelled halves of the same replicon will strongly underrate it. Moreover, if two neighbouring initiation points are far apart they will be less often identified as parts of the same fibre[11]. Therefore, although on a relative basis, centre–centre measurements do reflect the density of replicative units along DNA molecules, in absolute terms they are likely to provide a gross exaggeration of this parameter

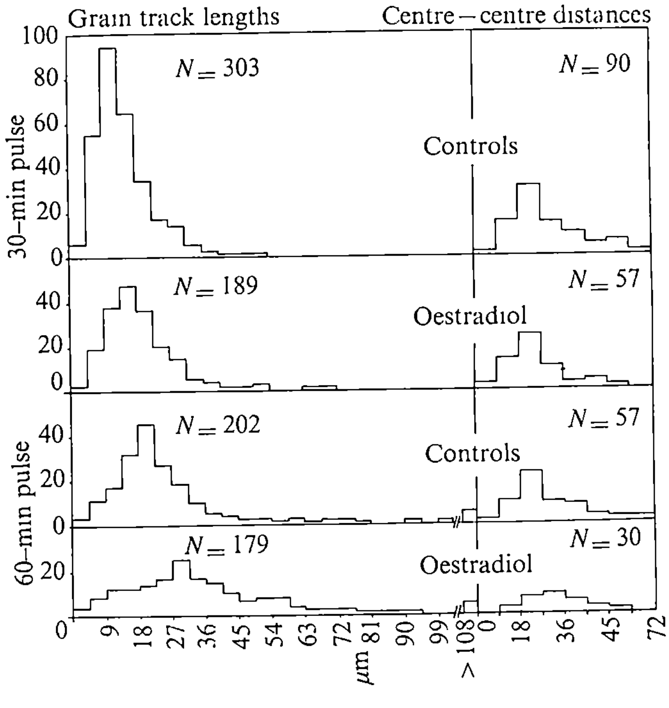
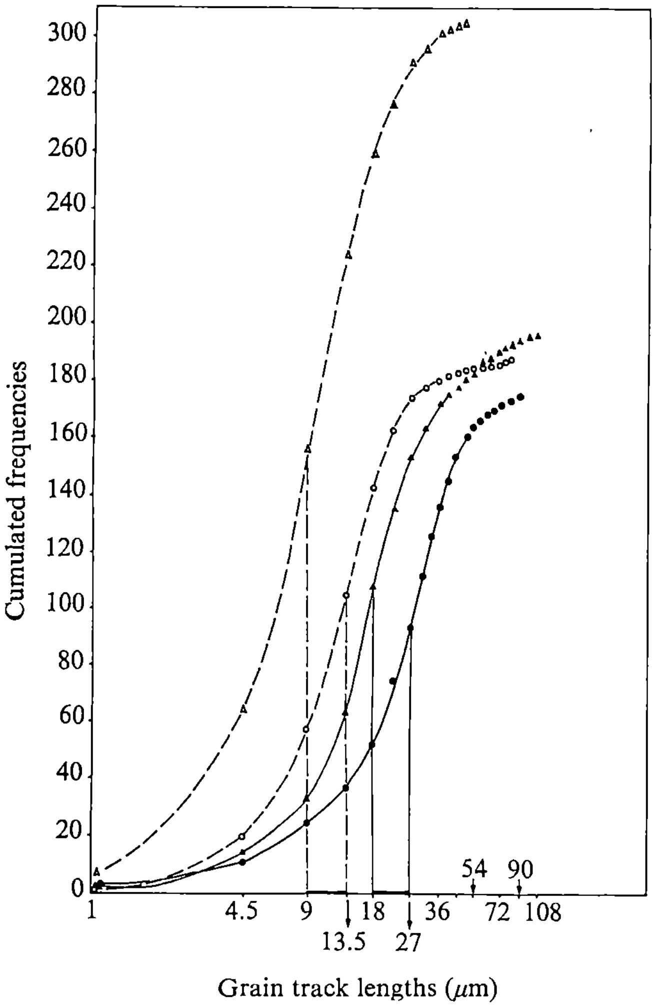


Fig. 1 Effect of oestradiol on lengths and distances between centres of ^{3}H-TdR labelled segments in DNA molecules isolated from mice uterine epithelial cells. One week after ovariectomy, albino mice were grouped into 10 experimental and 14 control animals which were injected intraperitoneally with 100 ng 17 β-oestradiol in 100 μl propylene glycol, respectively, or with the same volume of vehicle solvent alone. After 15 h oestrogen action the uterine horns were dissected out, split longitudinally and transferred into TC medium Eagle Earle BSS (Difco) at 929 mg % in water, neutralised at *p*H 7 3 by 5% $NaHCO_3$ and supplemented with calf serum (10 ml per 100 ml of initial solution). A first incubation period of 30 min at 37 °C was effected in the presence of 2×10^{-6} M 5-fluorodeoxyuridine (FUdR) only, to enable optimal DNA labelling[14]. Thereafter the uteri were pulse-labelled in the same medium containing FUdR but supplemented with ^{3}H-thymidine (^{3}H-TdR) of high specific activity (52 Ci mM^{-1}) at a final concentration of 5×10^{-6} M In each group half of the uterine horns were removed and washed after 30 min whereas the remaining material was incubated with the isotope for 60 min After termination of the ^{3}H-TdR pulse, uterine epithelial nuclei were isolated as described by Smith *et al.*[15] The nuclear pellets were resuspended in 0.3 ml of phosphate buffered saline (*p*H 7.4) and were used to perform DNA fibre smears according to the method of Lark *et al.*[16]. These smears were processed for autoradiography by dipping in 50% diluted Ilford K5 emulsion and were developed in 50% Dektol (Kodak) after 17 weeks' exposure. Measurements were performed with a micrometric scale only on internal autoradiograms to avoid broken DNA sections (that is by disregarding both end segments of tandem arrays on which measurements were effected) Statistical comparison was performed by use of Wilcoxon's rank test. With both pulse durations the increment of track lengths due to oestradiol was highly significant ($P<0.001$) whereas there was no significant difference between distributions of centre–centre distances.

Our data support the conclusion that oestradiol accelerates fork growing rate. The lengthening of labelled segments from experimental uteri does not seem attributable to an increased number of fusions between neighbouring units as, first, in the 30-min pulse histogram, at least, there is no significant increase compared with controls of long fused sections, and second, there is apparently no shortening of intervals which could account for a higher rate of fusion. On the contrary, when pulse labelling of oestrogen-treated material lasted 60 min, the longer size of labelled sections induced artificial lengthening of centre–centre distances as a result of more numerous fusions which are represented by the greater right-hand tail of the corresponding histogram of track lengths. There is, however, a possibility that in addition to increasing replication speed, oestradiol would induce a higher density of operating replicative units which could not be evidenced in our study because of the relative scarcity of centre–centre measurements.

Several authors[1–6] have proposed a reduction of S phase duration in uterine epithelial cells from oestrogen-treated mice ranging from 30 to 60%. In our study the 50% increase of fork growing speed accounts for a 33.3% shortening of S phase. Only a tentative estimate of fork growth rate can be derived from our data. The modal values which were found may be assumed to lie somewhere near unidirectional growth rate since many labelled sections correspond to half replicative units. Moreover, among replicons initiated during the pulse interval, only those in which DNA synthesis started during the first half of the labelling period will show continuous grain tracks longer than monodirectional segments. True fork growth rate should therefore be evaluated at about 0.3 μm and 0.45 μm per min in controls and oestradiol-treated cells respectively. Such values would fall within the range which has been observed in other mammalian cells of which DNA growth rate has been estimated to be around 0.5 μm min^{-1} (ref. 12).

Fig. 2 Cumulated frequency curves and modal values of labelled DNA lengths (log scale) in uterine epithelial cells from control and oestradiol-treated mice. Oestradiol produces a 50% increase in chain growth with both durations of ^{3}H-TdR pulse performed on uteri incubated in vitro. Controls: △, 30-min pulse; ▲, 60-min pulse. Oestradiol: ○, 30-min pulse; ●, 60-min pulse.



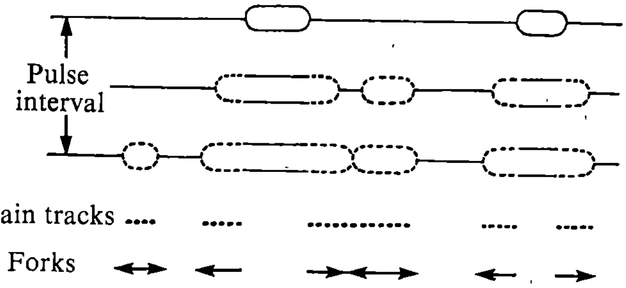


Fig. 3 Schematic example showing how different types of DNA labelled segments can be obtained after single pulse labelling with ^{3}H-TdR (labelled sections in dotted lines). Two replicative units were already operating before giving the pulse whereas two others were initiated at the beginning and at the end of the pulse interval (modified after Blumenthal *et al.*[13]).

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Troponin C in brain

The tropomyosin–troponin complex renders actomyosin from vertebrate skeletal muscle calcium sensitive. Troponin itself consists of three subunits: troponin I (TNI), troponin C (TNC) and troponin T (TNT)[1]. Pure TNI (24,000-dalton subunit) inhibits the actin–myosin interaction irrespective of calcium concentration. TNC (18,000-dalton subunit) binds calcium and releases the TNI imposed inhibition. TNT (37,000-dalton subunit) attaches the subunit complex to tropomyosin. Actin, myosin and tropomyosin have been isolated and characterised from a number of non-muscle cells[2,3]. We now describe the isolation and characterisation of a protein from chick embryo brain which is very similar to muscle TNC.

The isolation of this molecule from brain followed our observation that an 8 M urea extract of chick embryo brain contains a protein which migrates rapidly towards the anode in a 6 M alkaline urea acrylamide gel when 5 mM EGTA, a calcium chelator is present (Fig. 1*b*). The protein band has the same mobility as chicken skeletal muscle TNC (Fig. 1*c*). When 5 mM Ca^{2+} is added to the extract, the fast moving band disappears (Fig. 1*a*). This behaviour is similar to that of skeletal muscle TNC, which forms a complex with TNI in 6–8 M urea when calcium is present. EGTA blocks the complex from forming, allowing TNC to migrate rapidly in an alkaline urea gel, whereas the complex migrates much more slowly[4].

We isolated the most rapidly migrating band from a preparative alkaline urea slab gel of a chick brain urea EGTA extract, and analysed the protein by acrylamide gel electrophoresis. The isolated protein was composed of two major polypeptides with molecular weights of 21,000 and 17,000 (Fig. 2*a*). A sample of chicken skeletal muscle TNC (18,000 dalton) is shown in Fig. 2*b*.

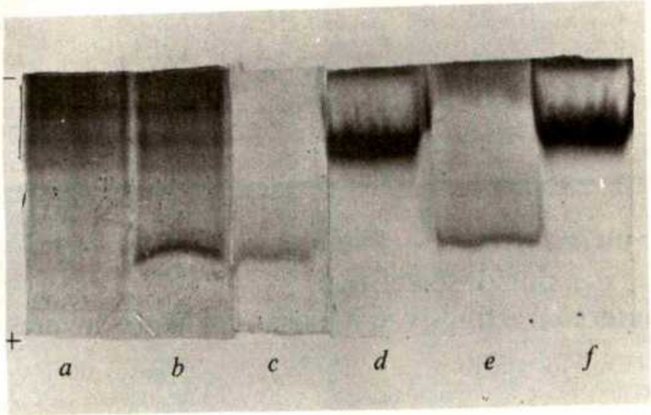

Fig. 1 Alkaline urea–acrylamide slab gels of proteins subjected to electrophoresis as described by Perry *et al.*[4] and stained with naphthalene black. One 14-d-old chick embryo brain from which all visible blood vessels were removed was placed in two volumes of 9 M urea. The brain was then homogenised by 15 strokes in a Dounce homogeniser with a tight pestle. The solution was then centrifuged for 30 min at 100,000*g* and the soluble extract divided into two parts. *a*, Electrophoresis of 0.3 ml of chick brain urea soluble extract in 5 mM $CaCl_2$; *b*, electrophoresis of 0.3 ml of extract in 5 mM EGTA; *c*, 5 µg chicken skeletal muscle TNC purified as described by Greaser and Gergely[1]; *d*, 5 µg of presumptive chick brain TNC and 15 µg of rabbit skeletal muscle TNI, purified as described by Greaser and Gergely[1], dissolved in 8 M urea, 5 mM $CaCl_2$ and electrophoresed; *e*, as in *d* except that 5 mM EGTA was substituted for $CaCl_2$; *f*, as in *d* except that 5 µg of chick skeletal TNC was used instead of the brain protein.

To obtain a pure protein, we used another property of TNC. In the absence of TNI, TNC migrates significantly more rapidly in a alkaline urea gel in the presence of Ca^{2+} than in EGTA[4]. We therefore combined the protein obtained from four EGTA preparative gels, added Ca^{2+} and ran another preparative gel. Again we eluted the most rapidly migrating band and analysed this protein by sodium dodecyl sulphate (SDS)-acrylamide gel electrophoresis. One protein band with an apparent molecular weight of 17,000 was obtained (Fig. 2*c*).

To determine whether the brain protein functions like muscle TNC, we substituted it for rabbit skeletal TNC in an actomyosin ATPase assay containing tropomyosin, TNI and TNT. The results shown in Table 1 indicate that the brain protein can restore calcium sensitivity to the muscle actomyosin ATPase, presumably by interacting with TNI, thereby relieving the inhibition induced by the tropomyosin–TNI–TNT complex, when calcium is present. More brain protein than muscle TNC is required, however, to relieve the inhibitory effect, and the resulting calcium sensitivity is lower than when the muscle TNC is used. It is possible that the affinity of the brain protein for muscle TNI is lower than that of the skeletal muscle TNC, or alternatively the brain protein may be partly denatured after the rather harsh purification procedure.

Following these assays the superprecipitates formed in the presence of Ca^{2+} were collected by centrifugation at 5,000*g* for 5 min. One such superprecipitate was analysed by SDS gel electrophoresis. Rabbit skeletal myofibrils containing the same amount of protein were also analysed (Fig. 2*d* and *e*). A protein band is present in the superprecipitate which comigrates with the brain presumptive TNC. This band is not present in myofibrils. This result suggests that the brain protein can bind to muscle actomyosin.

The brain protein was combined with excess rabbit skeletal TNI in 8 M urea and divided into two equal parts. Ca^{2+} (5mM) was added to one part, EGTA (5 mM) to the other and they were analysed by alkaline urea gel electrophoresis. When EGTA is present, a rapidly migrating protein, with the mobility of TNC is seen (Fig. 1*e*). When Ca^{2+} is present this band disappears and one sees a band which has the same mobility as a complex of rabbit skeletal TNI–TNC, and presumably represents a muscle TNI–brain TNC complex (Fig. 1*d* and *f*).

The amino acid compositions of chick embryo brain TNC and two chick muscle TNCs are shown in Table 2. TNC from skeletal and cardiac muscle has a characteristically high acidic/basic amino acid ratio and a relatively high content of phenylamine as does the brain protein.

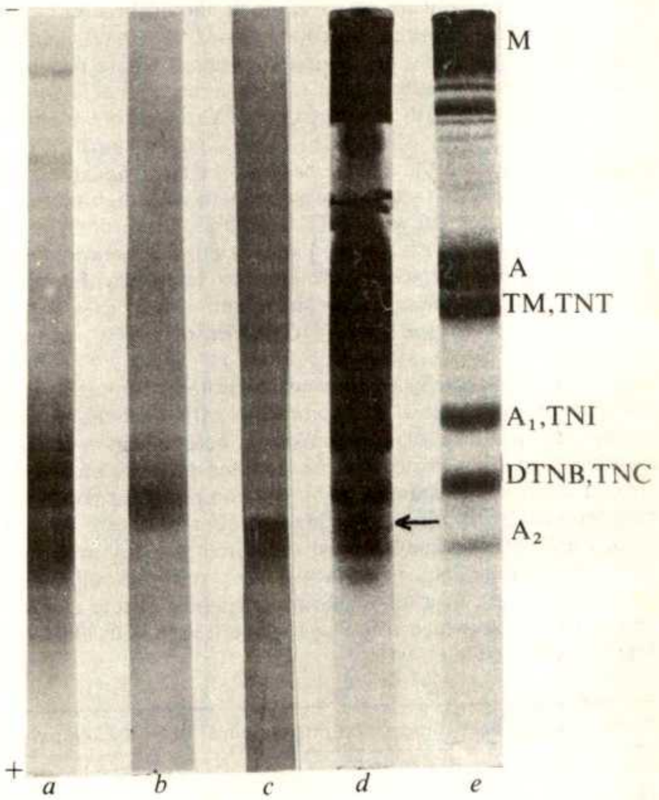

Fig. 2 10% SDS-acrylamide gels of proteins dissolved in SDS and subjected to electrophoresis as described by Weber and Osborn[8] and stained with Coomasie brilliant blue. *a*, 25 µg of protein isolated from preparative urea slab gel electrophoresis of 6 M urea soluble chick brain extract prepared as in Fig. 1 with 5 mM EGTA present; *b*, 5 µg of chick skeletal muscle TNC; *c*, The same protein as in *a* was dissolved in 6 M urea–5 mM $CaCl_2$ and subjected to preparative gel electrophoresis as in *a*. 10 µg of protein eluted from most rapidly migrating band is present; *d*, Ca^{2+}-induced superprecipitate elicited by chick brain TNC. Gel was intentionally overloaded to ensure that the rapidly moving bands were visible; *e*, 200 µg of rabbit skeletal muscle myofibrils. M, Myosin heavy chains; A, actin; TM, tropomyosin; TNT, troponin T; Al, highest molecular weight myosin light chain; TNI, troponin I; TNC, troponin C; DTNB, second heaviest myosin light chain; A2, lightest myosin light chain. This system does not resolve several components into separate bands.

Table 1 Effect of chick brain troponin C on the calcium sensitivity of rabbit muscle actomyosin ATPase in the presence of tropomyosin and troponin I and T*

Troponin subunits added	Mg-ATPase (μmol Pi/min/mg myosin) EGTA	Ca^{2+}	Calcium sensitivity $\left[1-\frac{\text{ATPase EGTA}}{\text{ATPase Ca}^{2+}}\right]\times 100$
a None	0.50	0.50	0
b Rabbit TNI-TNT	0.04	0.04	0
c Rabbit TNI-TNT + rabbit muscle TNC†	0.14	0.57	75
d Rabbit TNI-TNT +chick brain TNC†	0.06	0.11	46
e Rabbit TNI–TNT +chick brain TNC†	0.33	0.55	40

*Assays performed as described previously[9].

†In *c*, 0.08 mg rabbit skeletal muscle TNC added. In *d*, 0.09 mg chick embryo brain TNC added. In *e*, 0.20 mg chick embryo brain TNC added.

These results indicate that the brain contains a protein which is chemically and functionally similar to TNC. The molecular weight of the brain protein is slightly less than that of the muscle protein suggesting either that the two proteins are specified by different genes or alternately that the proteins are products of the same gene and the brain TNC is cleaved after synthesis, or during preparation. It should be noted that brain and other non-muscle tropomyosins also have smaller molecular weights than the muscle proteins, and are products of different gene classes[3].

Proteins similar to the brain troponin C are also present in growing chick sympathetic neurones, and in purified rat synaptic membranes prepared as described previously[5,6]. In both cases a urea extract contains a protein with high mobility in an alkaline urea gel with EGTA present. The protein band disappears when Ca^{2+} is added. Partially purified neuronal and synaptic membrane TNC-like proteins also interact with muscle TNI to form complexes stable in alkaline urea gels. These findings indicate that the brain TNC is at least partially neuronal in origin.

Puszkin and Kochwa[7] isolated a protein complex from synaptic membranes which combined with skeletal muscle myosin to produce a Ca^{2+}-sensitive actomyosin ATPase. Besides containing proteins corresponding to actin and brain tropomyosin in molecular weight, the complex also contained polypeptides with chain weights of approximately 38,000, 27,000 and 20,000. They suggest that these proteins represent TNT, TNI and TNC respectively. Our results confirm the presence in brain of a TNC-like protein and indicate the presence of a protein which is similar to TNI at least in its ability to interact with TNC.

Table 2 Amino acid composition of troponin C from chicken tissues

	Chicken cardiac*	Chicken skeletal*	Chicken brain†
Lys	13	11	10
His	1	1	0
Arg	4	6	7
Asp	25	25	25
Thr	8	7	8
Ser	5	6	4
Glu	30	31	29
Pro	3	1	3
Gly	13	12	10
Ala	8	12	12
Val	8	5	9
Met	9	10	5
Ile	8	10	7
Leu	12	9	13
Tyr	3	0	2
Phe	8	11	9

*Data from Hirabayashi and Perry[10].

†Analysis performed using the method of Stein *et al.*[11].
Results are in mol residues per 17,500 g.

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Subunit structure of human hexosaminidase verified: interconvertibility of hexosaminidase isozymes

Two major and several minor isozymes of hexosaminidase (Hex) exist in human tissues. In Tay-Sachs disease Hex A is missing but the activity of Hex B is increased. In Sandhoff's disease, both Hex A and Hex B are lacking; the residual activity represents increased amounts of a normal minor isozyme, Hex S. A fourth isozyme, Hex C, has an electrophoretic mobility very similar to that of Hex S, but seems to be unrelated genetically and structurally to A, B and S. The model which seems best to explain the relationship between Hex A, B and S is one in which they are polymers comprised of two different polypeptides which we have designated the α and β chains[1]. We have proposed that Hex B is a β-chain homopolymer, Hex A an α and β-chain heteropolymer and Hex S an α-chain homopolymer[2]. We now report that all interconversions between Hex A, B and S that are predicted by this subunit model can be performed.

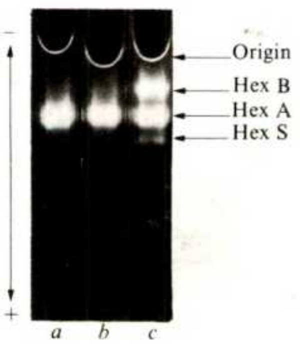


Fig. 1 Acrylamide disc electrophoresis of Hex. A: *a*, untreated; *b*, in buffered 3 M NaCl without freezing; and *c*, after freezing and thawing three times in buffered 3 M NaCl as described in the text. A 7.5% resolving gel in 0.375 M Tris-HCl, *p*H 8.9, polymerised with ammonium persulphate and TEMED, and a 2.5% concentrating gel in Tris (0.06 M)–H_3PO_4 (0.03 M), *p*H 7.2, polymerised with riboflavin were used. The upper buffer reservoir was Tris (0.052 M)–glycine (0.052 M), *p*H 8.9; and the lower reservoir was 0.1 M Tris–HCl, *p*H 8.1. Gels were run at 2 mA per tube for 2 h, and stained with 0.5 mM 4-methylumbelliferyl-N acetyl-β-D-glucosaminide in 0.25 M citrate–*p*H 4.4.

These studies were carried out with homogeneous preparations of human Hex A and Hex B, prepared by methods similar to those described previously[3], but with preliminary purification of the crude placental homogenate on concanavalin A Sepharose[4]. Reactivity with antisera was determined by precipitating enzyme with antibody in a mixture containing 0.04 M potassium phosphate *p*H 7.0, 0.012% bovine serum albumin (hexosaminidase-free), 20 μl of antisera[5] or control serum and 1–5 mU of enzyme. After incubating overnight at 4 °C, the mixture was centrifuged at 40,000*g* for 60 min and supernatant Hex activities were determined. Assays were carried out as before[3].

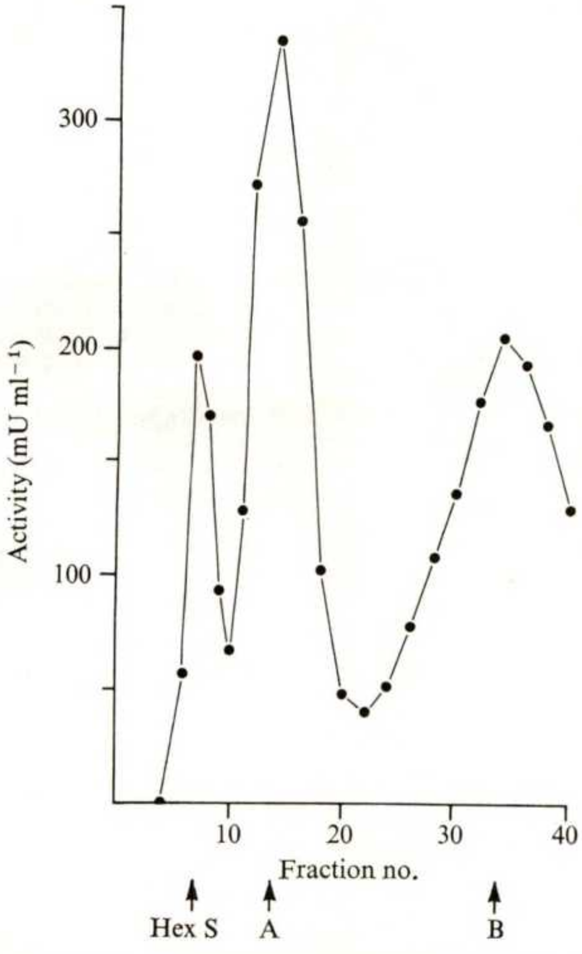


Fig. 2 Elution pattern from preparative polyacrylamide gel electrophoresis of Hex A which had been frozen and thawed in a high ionic strength salt solution. The system described for Fig. 1 was used with a Buchler Polyprep 200 apparatus with the lower buffer reservoir containing a fourfold concentrated buffer. Elution was performed with a 1:4 dilution of this buffer.

Homogeneous Hex A at a concentration greater than 4 U ml^{-1} could be converted directly into Hex B and an enzyme indistinguishable from Hex S by freezing and thawing in 3 M sodium chloride in 0.02 M potassium phosphate buffer, *p*H 7.0. After three or four cycles of freezing in a dry ice–acetone bath and thawing, the enzyme was submitted to acrylamide disc electrophoresis. As Fig. 1 shows, this treatment resulted in the appearance of bands of activity in the positions of Hex B and Hex S, which seemed to reach a maximum after four cycles of freezing and thawing. The formation of Hex B and Hex S could also be demonstrated on Cellogel electrophoresis. The isozymes found in a frozen and thawed preparation were separated on preparatory acrylamide disc electrophoresis (Fig. 2). The putative Hex S isozyme isolated by this procedure was reactive with anti-Hex A (83% enzyme removed) but showed little or no reactivity with anti-Hex B (14% enzyme removed), just as we had found with authentic Hex S obtained from tissues of a patient with Sandhoff's disease[2].

Since an equilibrium mixture between Hex A, B and S seemed to be formed on repeated freezing and thawing, it seemed possible that Hex A could be formed by freezing and thawing a mixture of Hex S and Hex B. As Fig. 3 shows, this was achieved using both the Hex S prepared from homogeneous Hex A (see above) and Hex S partially purified from the liver of a patient who had died of Sandhoff's disease. The Hex A formed by hybridisation of both Hex S preparations with Hex B was indistinguishable from placental Hex A by Cellogel electrophoresis and by DE 52 chromatography. No Hex A was found in a mixture of Hex B and S that was not subjected to freeze–thaw treatment, nor was any Hex A formed when Hex B or S were frozen and thawed separately.

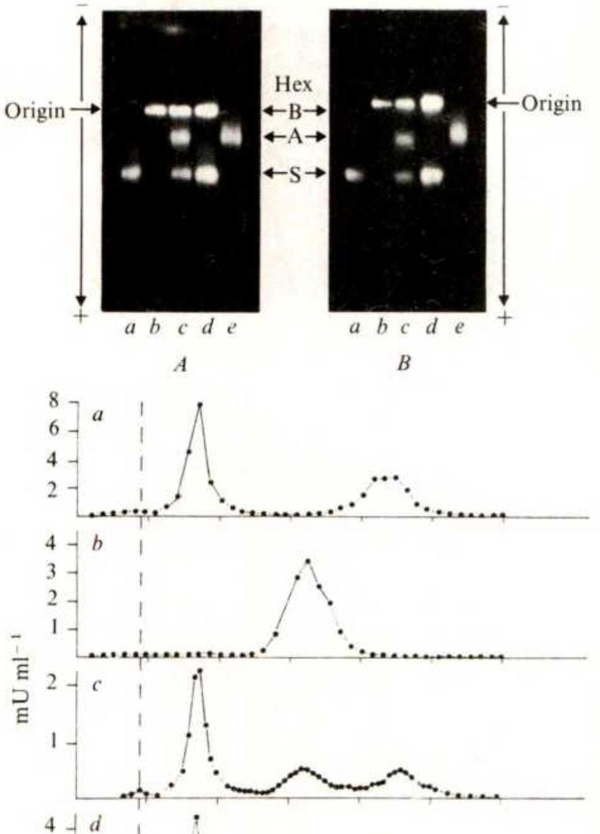
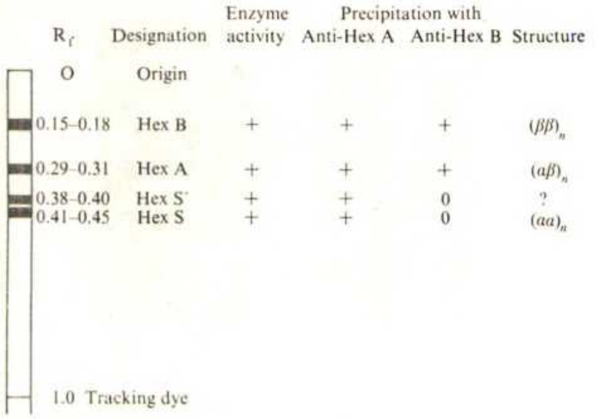


Fig. 3 *A*, Cellogel electrophoresis of: *a*, placental Hex S; *b*, placental hex B; and *c*, a mixture of placental Hex S and Hex B, all of which have been frozen and thawed three times in buffered 3 M NaCl. Channels *d* and *e*, are unfrozen controls of mixed placental Hex S and placental Hex B (*d*) and placental Hex A (*e*). The placental Hex S preparation was prepared from placental Hex A (as shown in Fig. 2). Electrophoresis was carried out for 2 h in a 20 mM potassium phosphate buffer, *p*H 7.0, at 92 V across the gel and 0.6 to 0.8 mA per cm. *B*, the same as (*A*) except that authentic Sandhoff's liver Hex S was used in place of Hex S prepared from placental Hex. A. *C*, Column chromatography elution on DE 52 (ref. 18) of: *a*, mixture of placental Hex S and placental Hex B which was not frozen; *b*, placental Hex A; *c*, placental Hex S and placental Hex B frozen and thawed three times in buffered 3 M NaCl; and *d*, Sandhoff's liver Hex S and placental Hex B frozen and thawed three times in buffered 3 M NaCl.

The close relationship between Hex A and Hex B has been evident for several years: both enzymes are missing in Sandhoff's disease and the two isozymes are strongly immunologically cross reactive. Models proposed to account for this relationship include the suggestions that Hex A is merely the neuraminic acid derivative of Hex B (refs 6 and 7) that Hex A and Hex B are conformers[8], that Hex A and Hex B are unrelated[9], and that Hex A and Hex B have a common subunit[10]. The results of attempts to distinguish between some of these alternatives by studying interspecific somatic cell hybrids have been confusing. Some studies indicated that the presence of Hex B was dependent on the presence of Hex A[11], some indicated that Hex A formation was dependent on the formation of Hex B[12], and others that Hex A and B are unrelated[9,13]. We believe that problems in interpretation of results of interspecific cell hybridisation arise because of difficulties in correctly identifying the enzymes in hybrid clones. Such difficulties are magnified in the case of glycoproteins, because the sugars attached to the protein may vary even from tissue within the same species[14].



R_f	Designation	Enzyme activity	Precipitation with Anti-Hex A	Precipitation with Anti-Hex B	Structure
O	Origin				
0.15–0.18	Hex B	+	+	+	$(\beta\beta)_n$
0.29–0.31	Hex A	+	+	+	$(a\beta)_n$
0.38–0.40	Hex S′	+	+	0	?
0.41–0.45	Hex S	+	+	0	$(aa)_n$
1.0	Tracking dye				

Fig. 4 Summary of properties and subunit structures of hexosaminidase isozymes

According to the model we have proposed[1], Hex A has a polypeptide structure of $(\alpha\beta)_n$, Hex B, $(\beta_2)_n$, and Hex S, $(\alpha_2)n$. Our model was derived from, and supported by, the following considerations: (1) the amino acid composition of homogeneous human Hex A and Hex B are different[15]; (2) Hex A contains an antigenic determinant not present in Hex B[1], but all the antigenic determinants present in Hex B are found in Hex A[1,16]; (3) Hex S contained only the antigenic determinants of Hex A and not those of Hex B[2,17]; (4) Hex A can be converted to Hex B and an alkylated α chain by treatment with merthiolate or *p*-hydroxymercuribenzoate[18] (not by neuraminidase, as had been claimed); (5) hybridisation of fibroblasts from Sandhoff's disease and Tay-Sachs disease produced complementation with the formation of Hex A[19]. According to our model, Tay-Sachs disease represents a mutation involving the α subunit, preventing formation of Hex A, $(\alpha\beta)_n$, and making available excess β chains, thus accounting for the increased levels of Hex B, $(\beta\beta)_n$. Conversely, Sandhoff's disease represents a mutation involving the β chain, preventing formation of Hex A and Hex B and making available excess α chains for the formation of the α-chain homopolymer, Hex S. The analogy between this model and the thalassaemias is evident: in α thalassaemia homopolymers of β chains form haemoglobin H, while free α chains are detected in β thalassaemia.

Our ability to interconvert Hex A, Hex B and Hex S demonstrates clearly the correctness of the subunit model. The properties and subunit structures of hexosaminidase isozymes are summarised in Fig. 4. Although we suggested previously that Hex S was probably a homopolymer $(\alpha_2)_n$, a heteropolymeric structure, $(\alpha\gamma)_n$ could not be ruled out in our earlier studies[2]. But the fact that Hex S as well as Hex B can be produced from homogeneous Hex A indicates that no subunits in addition to α and β are required to account for the formation of Hex A, B and S, and that Hex S is, indeed, an α-chain homopolymer.

On the other hand, Hex C, an enzyme which has been described in some detail by Dreyfus, Robinson and others[20] seems to be independent of the Hex A, B and S system. We have been able to show that the residual brain enzyme of our patient with Sandhoff's disease[2] was Hex C, and that it could easily be distinguished from Hex S on side-to-side comparison. We have confirmed the observation that this enzyme, from normal or Sandhoff's disease brain, is not affected by antisera produced against Hex A and Hex B, nor is it precipitated by an antiserum produced against Hex S. It seems likely that Hex C is a genetically independent protein. The minor isozymes, Hex I and Hex S′[2] are probably merely manifestations of the microheterogeneity of the carbohydrate portion of Hex B and Hex S, respectively.

Although the nature of the C, S′ and I isozymes require further study, the interconvertibility of Hex A, B and S verifies their proposed subunit structures. These findings are inconsistent with somatic cell hybridisation studies which assign totally distinct loci to Hex A and Hex B[9] or to the suggestion that the differences between Hex A and Hex B are merely conformational[8].

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Stepwise degradations and deamidation of the eye lens protein α-crystallin in ageing

KNOWLEDGE of the intracellular processes of ageing and breakdown of proteins is still very limited[1,2], but the extremely slow turnover of lens proteins in the eye provides a unique opportunity to analyse such processes. Throughout life fibre cells are formed from epithelial cells at the periphery of the lens, being successively covered by younger cells. Protein synthesis ceases soon after the differentiation into fibre cells is completed[3]. As a result the nucleus of the lens of an adult still contains the proteins formed during foetal life. Here we

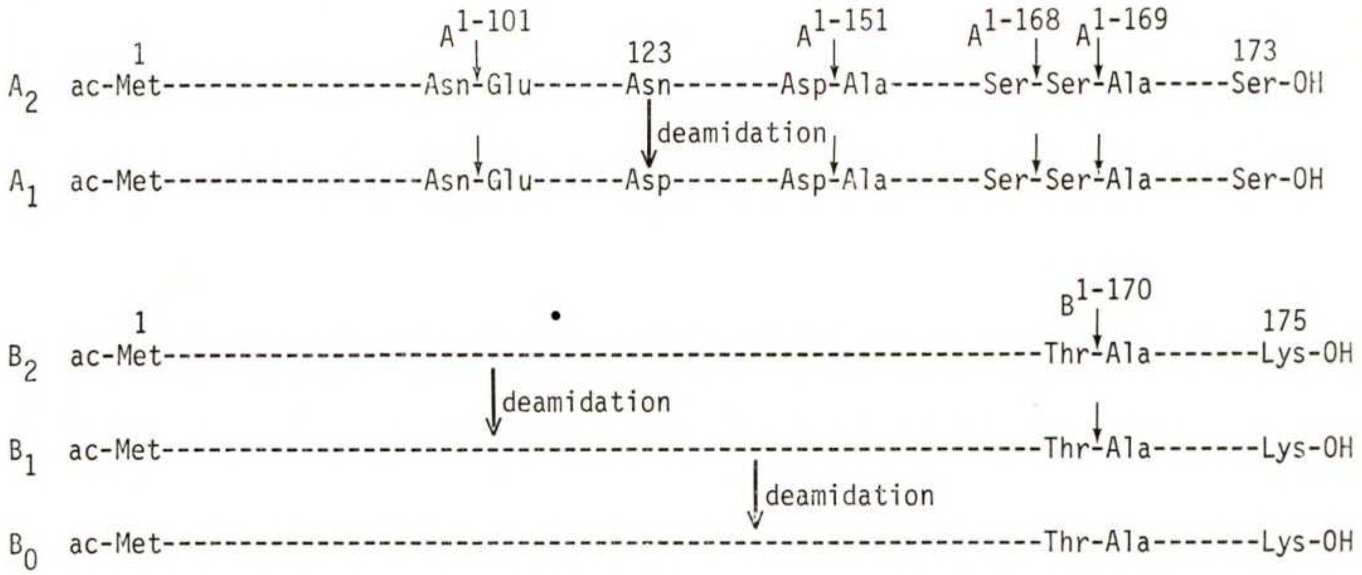
A^{1-101}
A^{1-151}
A^{1-168}
A^{1-169}
1
123
173
A_2 ac-Met----------------Asn-Glu------Asn-------Asp-Ala------Ser-Ser-Ala-----Ser-OH
deamidation
A_1 ac-Met----------------Asn-Glu------Asp-------Asp-Ala------Ser-Ser-Ala-----Ser-OH
B^{1-170}
1
175
B_2 ac-Met--Thr-Ala-------Lys-OH
deamidation
B_1 ac-Met--Thr-Ala-------Lys-OH
deamidation
B_0 ac-Met--Thr-Ala-------Lys-OH

Fig. 1 Polypeptide chains of bovine α-crystallin and their postsynthetic modifications. The newly synthesised A_2 and B_2 chains are 173 and 175 residues long respectively, showing 57% sequence homology[7,8]. Deamidation in A_2 of 123-Asn and possibly a second amide gives rise to A_1 (W. W. de J., F. J. van der Ouderaa and H. Bloemendal, unpublished). A^{1-169}, A^{1-168} and A^{1-151} are degradation products lacking the last 4, 5 and 22 residues of the normal A chain, and each occurs in two electrophoretic forms, corresponding to the amidated and deamidated state[9,14]. A^{1-101} lacks 72 residues from the C terminus and occurs in a single electrophoretic form[10]. B_1 and B_0 are most probably successive deamidation products of B_2, although the sites of deamidation have not been determined[10]. Removal of five residues at the C-terminal end of B_2 and B_1 gives rise to B_2^{1-170} and B_1^{1-170} (ref. 10).

demonstrate that differences in polypeptide structure observed between the cortex and nucleus[4–6] of the lens are due to an ageing process. Our data provide the first example of progressive degradation and deamidation of a protein, probably caused by non-enzymatic mechanisms.

The polypeptide chains of the major lens protein α-crystallin, a high molecular weight aggregate, are well characterised[7–10] (Fig. 1). It seems that all α-crystallin chains can be related to A_2 and B_2 by deamidation and degradation processes. The proportion of shortened chains increases from cortex to nucleus[10] (Fig. 2), suggesting an age-dependent degradation process, since no protein synthesis takes place any more in the inner cortex and nucleus. These data do not, however, exclude the possibility that the shortened chains are synthesised *de novo* in embryonic lenses, and are present in older nuclei as remnants of this embryonic stage. We therefore investigated the subunits of α-crystallin from prenatal lenses.

It has been shown previously that foetal bovine lenses contain mainly A_2 and B_2 (refs 11 and 12). In very young embryos two additional subunits have been observed[13] which we found by isoelectric focusing to correspond in position to two of the additional subunits of the older lens (Fig. 3*A*). The two additional embryonic subunits were isolated and analysed by tryptic digestion and peptide mapping, using previously described methods[7,8]. The peptide maps were identical to those of A_2^{1-151} and B_2^{1-170} isolated from calf nucleus. Analysis of embryonic α-crystallin by gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) revealed, moreover, the presence in large amounts of the A_2^{1-169} (or A_2^{1-168}) chain, which does not separate from normal A_2 on isoelectric focusing (Fig. 3*B*). Peptide mapping of a mixture of embryonic A_2 and presumed A_2^{1-169} confirmed it to be genuine A_2^{1-169} (ref. 14).

These findings show, therefore, that the shortened chains A_2^{1-169}, A_2^{1-151} and B_2^{1-170} are already present in embryonic lenses. Are these chains then the products of independent genes or postsynthetic derivatives of A_2 and B_2? The absence of A^{1-101} in embryonic lenses proves that it is an ageing product of normal A chains. For A_2^{1-168} and A_2^{1-169} it has already been demonstrated that they are degradation products of A_2 (ref. 14). Both A_2^{1-151} and B_2^{1-170} are present in higher proportions in adult nuclear α-crystallin than in embryonic α-crystallin, increasing from 7 to 25%, and from 5 to 15%, respectively.

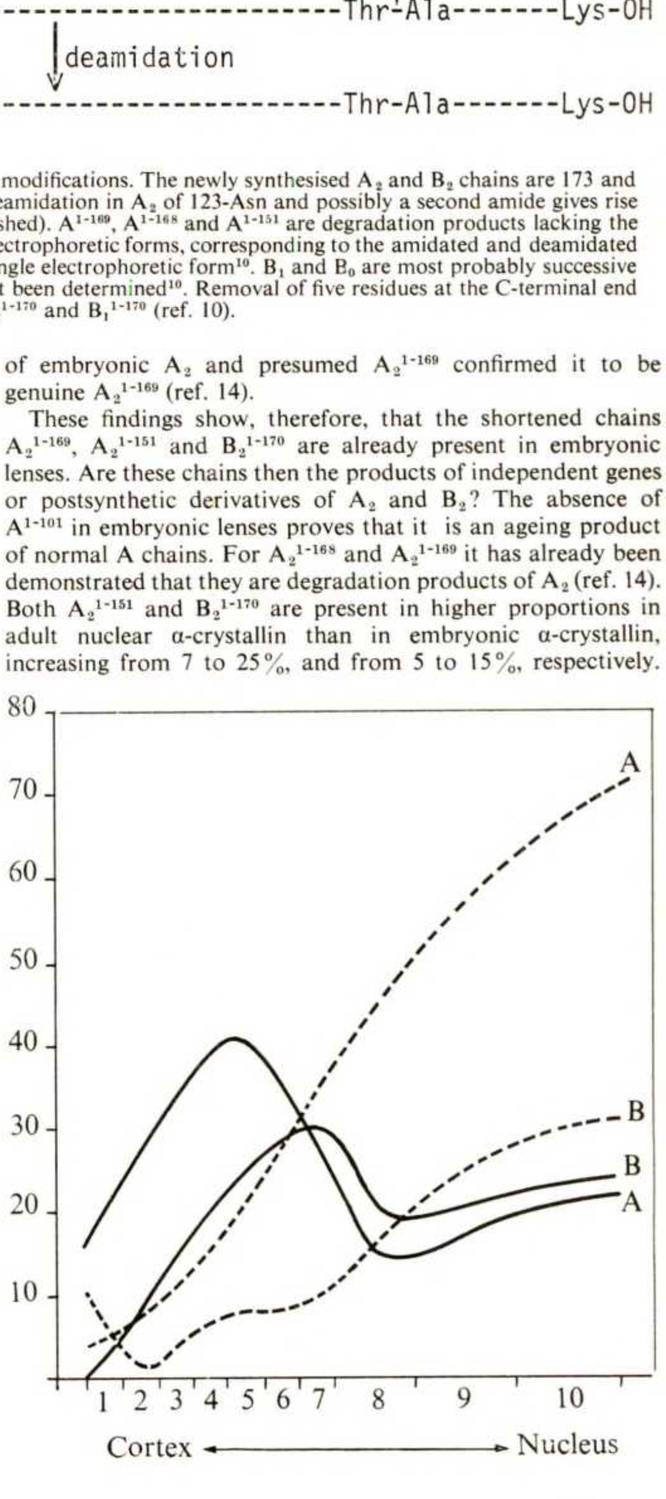

Fig. 2 Ratios of degraded (------) and deamidated (———) A and B chains in α-crystallin from successive concentric layers of calf lens (22 weeks old). α-Crystallin was isolated from ten concentric layers, corresponding to the numbers indicated on the abscissa, and analysed by acrylamide isoelectric focusing. The proportions of the different polypeptide chains were determined by scanning of the stained gels. The values on the ordinate express the percentage of degraded and deamidated A and B chains relative to the total amount of A and B chains, respectively.

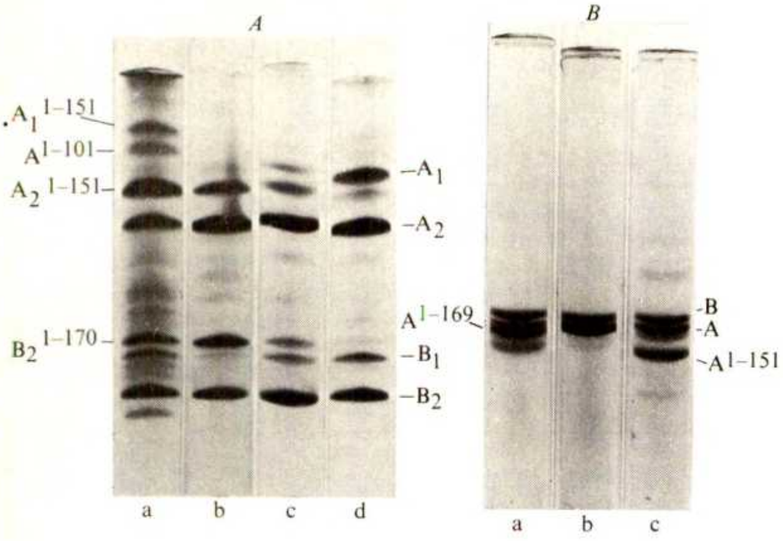

Fig. 3 *A*, Isoelectric focusing gels (*p*H 5–8) in 6 M urea of α-crystallin from: *a*, calf lens nucleus; *b*, 3-month-old foetus; *c*, 6-month-old foetus; *d*, calf lens cortex. *B*, SDS gel electrophoresis of α-crystallin from: *a*, 3-month-old foetus; *b*, calf lens cortex; *c*, calf lens nucleus.

This indicates that the formation of these chains continues in the adult nucleus, which is devoid of biosynthetic activity, and thus demonstrates the postsynthetic origin of these chains. Further proof comes from the fact that A^{1-151} and B^{1-170} can be produced *in vitro* simply by prolonged dialysis of α-crystallin against 0.1 M Tris-HCl buffer, *p*H 7.3 (ref. 15).

Our data thus show that the A_2 and B_2 chains are subjected to two types of postsynthetic modifications: deamidation and C-terminal degradation, as summarised in Fig. 1. The deamidation products A_1 and B_1 only appear in the later stages of foetal life and reach their highest level in the old bovine cortex, where a probable further deamidation product also becomes prevalent: B_0 (ref. 10). Postsynthetic deamidations have been demonstrated in other proteins, and can be obtained *in vitro* in proteins and synthetic peptides[16]. This deamidation process is non-enzymatic and its rate depends on the character of neighbouring residues[16].

The degradation of α-crystallin chains is apparently a postsynthetic process which starts early in embryonic life. The number of degraded chains is, however, clearly largest in the old bovine nucleus[10]. It seems, therefore, justified to consider these degradations as an ageing process. It seems that the chains are preferentially cleft at the sites indicated in Fig. 1. A stepwise breakdown, degrading the A chain successively to 169, 168, 151 and 101 residues, is suggested by the very high level of A_2^{1-169} and the absence of A^{1-101} in the embryonic lens, and the low level of A^{1-168} and high level of A^{1-101} in the old bovine nucleus[15].

Several features make it unlikely that the observed degradations are the result of enzymatic processes. The appearance of A^{1-101} is a very slow process, being most pronounced in the nucleus, which implies that a putative enzyme becomes active only very late after its synthesis. If the enzyme were an endopeptidase it should have a specificity (compare Fig. 1) unlike any known endopeptidase. If it were an exopeptidase it should have carboxypeptidase C-like activity to remove proline residues, and be delayed at the sites indicated in Fig. 1. The fact that degradations can be caused by prolonged dialysis of purified α-crystallin would require that the possible enzyme has the same molecular weight as α-crystallin or that the degradation is an autolytic process. Also the action of lysosomal enzymes cannot be involved, since no lysosomes seem to be present in the nucleus of the lens[17]. In conclusion we propose that the observed degraded chains are the result of non-enzymatic breaks of susceptible bonds.

The knowledge obtained from the age-related phenomena in eye lens proteins may contribute to a better understanding of ageing in general; although it can be questioned whether the processes taking place in the ageing lens are representative of ageing in other types of cells. In addition, the knowledge of normal ageing of lens proteins is of great interest to the study of those forms of cataract which are considered to be the result of precocious ageing[18].

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Erratum

In the article "Discrimination between eukaryotic and prokaryotic, and formylated and non-formylated, initiator tRNAs by eukaryotic initiation factor EIF–3" by I. G. Wool and R. S. Ranu (*Nature*, **257**, 616; 1975), the first four lines should read

PROKARYOTIC and eukaryotic cells contain two methionine accepting species of transfer RNA, $tRNA_f^{Met}$ and $tRNA_m^{Met}$ (refs 1–3). The formulated $Met\text{-}tRNA_f$ ($fMet\text{-}tRNA_f$) in prokaryotes[4] and non-formylated $Met\text{-}tRNA_f$ from . . .

and not as printed.

matters arising

Sympathoadrenal medullary activity in young, spontaneously hypertensive rats

INCREASED levels of serum dopamine-β-hydroxylase (DBH) have been reported in spontaneously hypertensive rats (SHRs)[1]. Although DBH is released from sympathetic nerve endings along with noradrenaline, levels of the enzyme in plasma do not seem to reflect accurately the degree of sympathoadrenal activity[2]. Plasma levels of noradrenaline mostly reflect sympathetic neuronal activity whence adrenaline (A) levels are indicative of adrenal medullary discharge. The content of catecholamines and biosynthetic enzymes in the adrenal are presumably indices of the activity of the adrenal medulla in synthesising and releasing catecholamines. The possible role of the sympathoadrenal system in initiating the increase in blood pressure in SHRs was examined by measuring levels of adrenaline and noradrenaline in the plasma and adrenal glands at the age of 4 weeks, the age at which the blood pressure of SHR begins to rise[3]. Normotensive rats of the Wistar Kyoto strain (WKY) from which the SHRs were derived were used as controls. Activities of adrenal tyrosine hydroxylase (TH), DBH, and phenylethanolamine-N-methyl transferase (PNMT), as well as plasma DBH, were also examined.

Table 1 Catecholamine concentrations* and dopamine-β-hydroxylase activity* in the plasma of 4-week-old spontaneously hypertensive and normal rats

	WKY	SHR
Total catecholamines	11.9±0.07	13.2±0.8
Noradrenaline	1.8±0.14	2.93±0.35‡
Dopamine-β-hydroxylase	7.84±0.24	13.4±0.6†

* Results expressed as ng of catecholamines, ng of noradrenaline and units of enzyme per ml of plasma, and are the means (± s.e.m.) for groups of 14 animals.

† $P < 0.001$ compared with control animals.

‡ $P < 0.01$ compared with control animals.

Table 2 Catecholamine content* and biosynthetic enzyme activities* in the adrenal glands of 4-week-old spontaneously hypertensive and normal rats

	WKY	SHR
Tyrosine hydroxylase	11.9±0.48	8.67±0.24‡
Dopamine-β-hydroxylase	25.6±2.6	17.7±0.92‡
Phenylethanolamine-N-methyl transferase	8.43±0.18	5.13±0.12†
Adrenaline	2.15±0.15	1.70±0.09§
Noradrenaline	0.85±0.10	0.75±0.04

* Results are expressed as ng of catecholamines, ng of noradrenaline, and units of enzyme per mg of adrenal and are the mean values (± s.e.m.) for groups of 14 animals.

† $P < 0.001$ compared with control animals.

‡ $P < 0.01$ compared with control animals.

§ $0.02 < P < 0.05$.

Male rats, 4 weeks old and weighing 70–90 g, were decapitated, and the first 1.5 ml of blood, which was ejected in a pulsatile stream from the trunk, was collected in heparinised tubes kept on ice. After centrifugation at 6,000*g* for 20 min at 4 °C, an aliquot of the plasma was removed for an assay of the DBH activity[4]. The proteins in 400 µl of the plasma were precipitated by the addition of 10 µl of 40% perchloric acid, and the mixture was kept at −10 °C until it was assayed for total catecholamines (adrenaline+noradrenaline)[5] and noradrenaline[6,7]. The catecholamine content of the adrenals was assayed fluorimetrically[8], and the TH (ref. 9), DBN (ref. 4), and PNMT (ref. 10), activities were assayed radiometrically.

In young SHRs the plasma levels of both the noradrenaline and the DBH were increased over those of the WKY (Table 1) but the total catecholamines (mostly A) were not significantly different. In contrast to the increased TH and DBH activities in the adrenal glands of adult SHR[11], the activities of TH, DBH, and PNMT in the adrenals of 4-week-old SHR were decreased (Table 2). Furthermore, the A content of the adrenals of the SHRs were lower than those of the WKY.

These observations are consistent with the view that, at 4 weeks of age, sympathetic neuronal activity in SHRs is increased, whereas adrenal medullary activity is depressed. Adrenal medullary activity is increased when sympathetic nerves are destroyed by 6-hydroxydopamine[12] and it is likely that depressed adrenal medullary activity may be secondary to the increased sympathetic neuronal activity. The exaggerated sympathetic neuronal activity at that age may be an essential factor in the course of development of hypertension in SHRs.

During this study, H.G. was supported by the Deutsche Forschungsgemeinschaft, Bonn, West Germany. J.M.S. is a visiting scientist at the National Institute of Mental Health, and M.F.R. is a research associate in the Pharmacology–Toxicology Program of the National Institute of General Medical Sciences.

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NAGATSU REPLIES—The findings of Grobecker *et al.*[1] agree with previous results[2] on increased serum DBH of SHRs at 3 weeks of age, and are consistent with the view that sympathetic neuronal activity in young SHRs is increased. As additional evidence, I have found that DBH activity in the mesenteric vessels of SHR at 3 weeks of age is increased, indicating increased sympathetic neuronal activity in blood vessels. Interestingly, DBH activity in the locus coeruleus area of the brain is decreased[3].

In contrast, serum DBH activity and tyrosine hydroxylase (TH) and DBH activities in blood vessels of SHRs at 14 weeks of age do not differ significantly from those of normotensive Wistar–Kyoto rats[4], whereas TH and DBH activities in adrenal glands are increased[4,5]. These results suggest that the increased sympathetic neuronal activity in young SH rats is decreased during the development and establishment of hypertension in adult SHRs and whereas the adrenal medullary activity in adult SHRs is increased.

The results of Grobecker *et al.* on decreased TH, DBH, and phenylethanolamine N-methyltransferase (PNMT) in the adrenal glands of SHRs at 4 weeks of age are of interest and indicate depressed adrenal medullary activity in contrast to increased sympathetic neuronal activity. I have, however, observed a slight but significant increase of DBH activity in the adrenal glands of SHRs at 3 weeks of age[3].

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Can supernovae make white dwarfs?

LEVENTHAL and McCall[1] have presented a model for a type I supernova light curve, in which the two sections of exponential decay of the light curve are explained by the accelerated decay of $^{56}\mathrm{Ni} \rightarrow {}^{56}\mathrm{Co} \rightarrow {}^{56}\mathrm{Fe}$, occurring in a fully convective white dwarf which is formed by the supernova implosion–explosion. While they did give reasons why a type I supernova would be expected to synthesise ^{56}Ni, they failed to discuss whether it would produce a white dwarf, a result which is strongly, though not entirely, unsupported by recent calculations.

The problem centres on whether the ^{12}C-detonation model of a supernova, thought to apply to stars in some subrange of 4–$8M_\odot$, can leave a white dwarf, or, indeed, any collapsed remnant. It is already clear (ref. 2 and W. D. Arnett, unpublished) that stars more massive than $\sim 8M_\odot$ all undergo core collapse and produce neutron stars or black holes.

Buchler[3] has assumed various arbitrary central densities, higher than those now thought to be attainable, for half ^{12}C, half ^{16}O, $1.4M_\odot$ degenerate cores and calculated the results for carbon detonations at these densities. At central density $\rho_c = 8.6 \times 10^9$ g cm^{-3}, the star was completely disrupted. In a small range around $\rho_c = 1.6 \times 10^{10}$ g cm^{-3}, his calculation did in fact produce an extended $1M_\odot$ Ni–Fe white dwarf, with the remaining $0.4M_\odot$ being blown off. At $\rho_c = 2 \times 10^{10}$ g cm^{-3} a neutron star remnant was produced.

Couch and Arnett[4] have recently carried out further calculations, using the ^{12}C detonation model and including all effects yet thought of, such as a convective Urca shell, which might postpone carbon ignition and allow the high central densities necessary for collapsed remnant formation to be achieved. Their models disrupted completely, except for the case where the C–O core was initially only 10% C by mass. The carbon burned non-explosively and a remnant could possibly be formed unless the central density reached a critical density (between 1×10^{10} and 2×10^{10} gm cm^{-3}), where the oxygen would ignite. They point out however that if He burning had produced such a low C–O ratio, it might become impossible to understand the nucleosynthesis of C and O, which is, presumably, done in higher mass stars, which are able to preserve and eject these nuclei.

Thus the formation of white dwarfs by supernovae remains highly problematical. If it could be shown to occur, Leventhal and McCall's model of type I supernova light curves would gain much credibility. It could also frustrate attempts (ref. 5 and B. M. Tinsley, unpublished) to deduce the mass below which, stars are able to shed enough mass by the planetary nebula mechanism to become stable white dwarfs, by counting white dwarfs in open clusters with high-mass turn off points on the main sequence.

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Carotid body chemoreceptors

THE "New theory for receptor mechanism of carotid body chemoreceptors" proposed by Osborne and Butler[1] requires that the rate of dopamine secretion from the glomus cells be high when blood gas tensions are normal and reduced in hypoxic conditions. The results of new experimental studies[2,3] are incompatible with the 'new theory' because the relationship between secretion of carotid body catecholamines and blood oxygen was found to be opposite to that postulated. In one study[2], the formaldehyde-induced fluorescence of the glomus cells was measured to monitor changes in their catecholamine content. In untreated cats, hypoxia resulted in higher fluorescence intensity of type I cells from innervated carotid bodies than from denervated ones. When cats were treated with a decarboxylase inhibitor, intensity of fluorescence was decreased in innervated carotid bodies compared with denervated organs after hypoxia. Sampson *et al.*[2] concluded that an increase in centrifugal impulse activity in the sinus nerve induced by hypoxia enhanced both release and synthesis of catecholamines in the carotid body. In the second study[3], the content of noradrenaline plus adrenaline in the cat carotid body was measured by the trihydroxy indole method. The content of these amines in the carotid body decreased after hypoxia and increased above the air control level when inspired oxygen concentration was raised to 40%. The decrease in catecholamine content was greatly attenuated when the carotid sinus nerve was cut before hypoxia.

Since the fluorescence technique used measured dopamine as well as noradrenaline and adrenaline, the increase in fluorescence in untreated carotid bodies after hypoxia may be attributed to enhanced synthesis of dopamine, which serves as precursor for noradrenaline and adrenaline. This is the case in the adrenal medulla where neurogenic stimulation activates tyrosine hydroxylase and increases dopamine content while releasing noradrenaline and adrenaline[4]. The results of the two carotid body studies[2,3] are consistent with the idea that stimulation of efferent neural pathways to the carotid body during hypoxia enhances both synthesis and release of catecholamines. The data provide no support for the 'new theory'[1] which requires that hypoxia depress secretion from the glomus cells of the carotid body and postulates that secretion is regulated independently of sinus nerve efferent pathways.

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OSBORNE AND BUTLER REPLY—Our conclusion[1] that a catecholamine (probably dopamine) is continuously released from the glomus cells during normoxia, and that the rate of release decreases during hypoxia, was based on the following: (1) glomus cells store large amounts of catecholamines, in particular, dopamine[2]; (2) catecholamines, especially dopamine, suppress or block the chemoreceptor discharge[3,4]; and (3) α-adrenoreceptor blocking agents greatly increase receptor firing rate (up to tenfold)[3,4] during normoxia and the effectiveness of the these blocking agents decreases during hypoxia[3].

It has since been indicated that there is a high turnover rate of catecholamines in the glomus cells, and that it seems likely that these cells release dopamine at a high rate[5]. This catecholamine activity by the glomus cells is envisaged to occur in normoxic conditions. Indeed, the metabolism of catecholamines requires the presence of molecular oxygen[6]. These observations suggest that as the oxygen availability decreases, the metabolism of catecholamines declines.

In the above article[7], the phrase "the increase in fluorescence in untreated carotid bodies after hypoxia" is puzzling in view of the facts that Mills and Slotkin[8] describe a decrease in catecholamine content of the carotid body during hypoxia, and Sampson *et al.*[9] did not in fact make the straightforward comparison between the amounts of catecholamines present in the glomus cells of untreated normoxic and hypoxic carotid bodies. We do not therefore accept that these two papers[8,9] contain unequivocal evidence regarding the effect of hypoxia on the release of catecholamines from the glomus cells of the carotid body.

Furthermore, Mills and Slotkin[8] state that the decrease in catecholamine content of the carotid body during hypoxia is largely mediated by way of the sinus nerve, whereas Sampson *et al.*[9] report that the catecholamine content of the glomus cells of the carotid body was higher in those organs with an intact sinus nerve. In an attempt to reconcile these apparent differences, Mills *et al.*[7] state more precisely that Mills and Slotkin[8] were referring to noradrenaline and adrenaline only, whereas Sampson *et al.*[9] measured total catecholamines. They now conclude that dopamine levels are increased within the glomus cells, whereas noradrenaline and adrenaline are released in response to efferent nerve activity. Whether this effect is mediated through a direct supply to the glomus cells or via the vasculature is not discussed by these authors, but it does raise the important issue regarding which catecholamine(s) is the major agent of chemoreceptive inhibition.

As has been shown[5,10,11], and as pointed out here, dopamine seems to be the favoured candidate.

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Spectacle haloes

THE nuclear geophysical enigma of the ^{210}Po haloes[1] is quite fascinating, but the explanation put forward on the basis of a novel form of ^{206}Pb is not easy either to understand or to believe.

If no primordial lead is present, all of the ^{206}Pb, ^{207}Pb and ^{208}Pb found in ordinary U–Th haloes will be derived from polonium α decay. To explain all of the polonium haloes observed by Gentry[1–3] without apparent α-emitting precursors it is not useful to assume new types of stable lead daughter products but rather some unknown, long lived isomers either of ^{218}Po and ^{210}Po or of β-emitting precursors of these. The haloes may be explicable without such an assumption. What is needed is that in a usual halo the polonium isotopes must decay while still in the uranium inclusion from which they are derived, whereas in the enigmatic halo they must together have migrated by at least hundreds of micrometres from their ancestral uranium.

Since the enigmatic halo has an intensity comparable with that of an ordinary halo, it must be attributable to the decay of quantities of polonium corresponding to the total output of a normal, halo-producing, uranium inclusion for at least tens of millions of years. That is a very long period compared with the half lives of isotopes of any elements other than uranium and thorium themselves. Conditions must, therefore, have been such as to allow migration from the original parent sources for very long periods.

The most likely migrant would seem to be lead, since emanation or polonium would give larger haloes than are seen. In that case there would be ^{210}Pb with a half life of 22 yr in one series and ^{211}Pb with a half life of 38 min in the other. Further, the ^{232}Th series, which eventually produces the observed ^{208}Pb, has ^{212}Pb with a half life of 10.6 h as a carrier, though it has no polonium with a half life of longer than 0.16 s. I do not know the temperature required for that migration of lead at a suitable rate, but it is, presumably, fairly high. Geological evidence would be interesting: Gentry himself has described[2] short fission tracks which suggest that some mica matrices may have been at a high temperature for a long time.

I do not claim that this explanation is highly probable—the events to be explained are rare—but Gentry's explanation would seem to require a higher order of improbability. Both ^{218}Po and ^{210}Po would need to have unknown precursors with half lives of a few hundred million years; lead or bismuth precursors would presumably have crystallised out with observable quantities of stable isotopes of the same elements. Finally, Gentry has also found[2,3] a variety of dwarf or other haloes for which he assumes different, otherwise unknown, α-emitters. All of those α-emitters apparently have mutually similar half lives or they would either have decayed too soon or would still be around in observable amounts. One such unknown isomer would be unlikely enough; so many would seem very close to impossible.

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GENTRY REPLIES—The crucial question is whether the polonium atoms which formed the polonium haloes were secondarily derived from the decay of uranium and thorium (see, for example, ref. 1) or from unknown isomers[1], or whether they were primordial.

The isomer hypothesis[1] is one way that polonium haloes could have been explained. My present view is that experimental results have ruled out the isomer hypothesis.

As for the postulate that uranium daughter activity provides a source of polonium atoms, I agree that in special cases polonium haloes can form in that manner: hundreds of such secondarily formed ^{210}Po haloes have been found[2] in a uranium-rich coalified wood from the Colorado Plateau. Earlier, when uranium-bearing solutions interacted with the uncoalified wood, numerous sites were formed containing a variety of elements. Uranium haloes subsequently developed around uranium-rich sites and ^{210}Po haloes around Pb–Se inclusions. The absence of ^{214}Po and ^{218}Po haloes means that even though the hydrothermal transport was high enough to permit the accumulation of ^{210}Po and ^{210}Pb to form ^{210}Po haloes, it was too low for many of the nuclides with shorter half lives—^{214}Pb, ^{214}Bi, ^{214}Po and ^{218}Po—to accumulate before decay. Thus, the formation of ^{214}Po and ^{218}Po haloes was inhibited.

Acceptable as that explanation is as far as ^{210}Po haloes in coalified wood are concerned, there are formidable obstacles in using it to account for the variety and distribution of ^{210}Po, ^{212}Po, ^{214}Po haloes in minerals. Except along conduits or cracks where solutions have passed, atomic migration in minerals proceeds by diffusion. Since the r.m.s. distance, x, that an atom of diffusion coefficient, D, will migrate in time, t, is $x = (2\,Dt)^{1/2}$ and the maximum value of D_{Pb} (at 20 °C) in 7 different minerals[3] is 10^{-18} cm^2 s^{-1}, a ^{210}Pb atom (with a half life of 22 yr) would migrate, on average, about 10^{-5} cm before decay. Even at 1,038°C the D_{Pb} value (10^{-11} cm^2 s^{-1}) in microclinepertite[3] yields negligible migration ($x = 0.11$ cm) of a ^{210}Pb atom before decay. Further, this is a somewhat unrealistic case, for even though shortened fission tracks exist in some micas[4], the great majority of minerals containing polonium haloes show no evidence of high temperature episodes. Concerning Po haloes in fluorite I can be very specific. The D_{Ba} ($\simeq D_{\mathrm{Pb}}$) value is less than 10^{-22} cm^2 s^{-1} at 300 °C (ref. 5) and it is certain that the fluorite has not exceeded that temperature during or since halo formation, because halo coloration disappears within minutes in this temperature range[6]. In that case a ^{210}Pb atom would migrate only about 10^{-7} cm before decay.

Thus, if Po or Pb radionuclides were migrating to Po halo radiocentres, most would have decayed in transit and produced a large excess of α-recoil tracks close to the radiocentres, which is contrary to observation[7]. An equally strong objection to the uranium-daughter hypothesis in uranium-poor (p.p.m. or less) minerals is that many Po haloes (such as the 'spectacle' halo) are located in the interior of large pegmatitic crystals as well as in small granitic mica flakes where they are often more than 10 cm and sometimes much less than 100 cm away from a significant uranium source. The fact that ^{214}Po and ^{218}Po haloes often occur profusely in some minerals (10^3–5×10^4 haloes cm^{-3}), whereas their formation was inhibited even in the uranium-rich (16% U) coalified wood where nuclide transport rates were comparatively high, certainly suggests some other origin for polonium haloes in minerals.

But if isomers and uranium-daughter diffusion did not produce polonium haloes in rocks, we are left with the idea that polonium haloes originate with primordial Po atoms just as U and Th haloes originate with primordial ^{238}U and ^{232}Th atoms. Thus, a new type of Pb exists in polonium halo radiocentres, derived specifically from primordial polonium α-decay. Carried to its ultimate conclusion, this means that polonium haloes, of which there are estimated to be more than 10^{15} in the Earth's basement granitic rocks[8], represent evidence of extinct natural radioactivity, and thus imply only a brief period between 'nucleosynthesis' and crystallisation of the host rocks.

If this conclusion is considered highly improbable, then another explanation for the origin of the polonium should exist.

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Microorganisms on manganese nodules

WE report here tubular forms found on the outer surface of a piece of coral coated with manganese from the eastern flank of the Mid-Atlantic Ridge (biotrawl at 747 m, V22-D6 Lamont–Doherty Geological Observatory). Scanning electron microscopy (SEM) of a dark zone of the coral revealed well preserved coiled tubular fragments which may be *Saccorhiza* averaging 2–3 μm in diameter (Fig. 1*a*–*d*). These tubular structures were not found on the inner surfaces. They are, however, ubiquitous throughout the dark ferromanganese zones on the outside of the manganese-coated coral. They seem merely to adhere or be cemented to zones rich in ferromanganese which show little distortion (Fig. 1*b*–*c*). We suggest that these organic structures are better preserved on fragments coated with ferromanganese than in the interior of compacted ferromanganese modules such as those studied by Greenslate[1].

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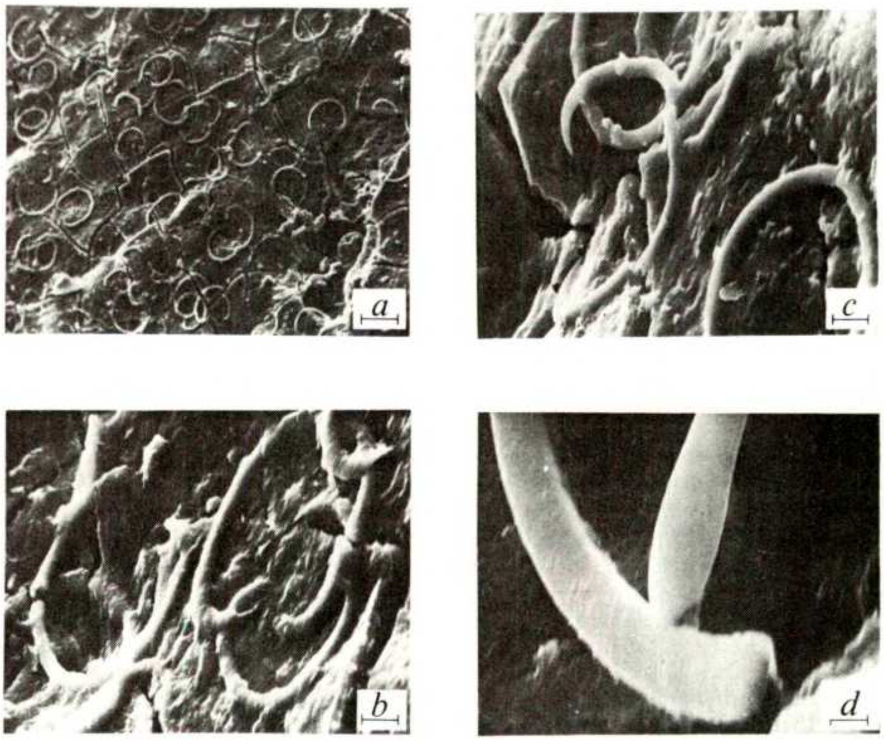

Fig. 1*a*, SEM of the outside region of manganese-coated coral (V22-D6), showing circular, tubular structures (scale bar, 80 μm); *b* and *c*, SEM of circular structures showing their adherence (*b*) and cementation (*c*) to the surface and their tubular nature (scale bar, 8 μm); *d*, SEM of tubular structures 2–3 μm in size (scale bar, 1.6 μm).

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reviews

Outweighing bones of contention

By any standards, the life and career of Louis Leakey was extraordinary. Kenyan by birth, Kikuyu by adoption and anthropologist by education he was still more: contemporary historian, palaeontologist, graphologist, museum curator, Criminal Investigation Department investigator, zoologist. But in this age of specialisation he was no less remarkable for his diversification than for his accomplishments, as these two books amply demonstrate.

By The Evidence, the second instalment of Louis Leakey's memoirs, covers the years from 1932 to 1951 and follows the initial volume, *White African,* which dealt with his first thirty years. *By The Evidence* encompasses a period of remarkable and varied productivity but also of serious professional conflict and controversy. These are the years of the initial explorations at Olduvai Gorge, Rusinga Island and other sites, the first Pan-African Congress, the discovery of *Proconsul;* they are also the years of stinging criticism and debate over his claims for the fossil hominids from Kanam and Kanjera. This was also a period of active involvement with police investigations in Kenya and of the development and expansion of the Coryndon Museum in Nairobi into what eventually became the National Museum of Kenya. The book eloquently describes his concern for the vanishing cultures and languages of East Africa; it also documents his efforts and ultimate frustrations in preserving something of the past for the future. His three-volume, 700,000-word monograph on the Kikuyu is still unpublished, although funds from the Leakey Foundation may soon solve this problem.

By the Evidence: Memoirs, 1932–1951. By L. S. B. Leakey. Pp. 276+12 photographs. (Harcourt Brace Jovanovich: New York and London, January 1975.) £2.95.

Leakey's Luck: The Life of Louis Seymour Bazett Leakey, 1903–1972. By Sonia Cole. Pp. 448+50 photographs. (Collins: London, May 1975.) £5.50.

Louis with man-eating crocodile on Rusinga Island, Kenya

Sonia Cole's excellent book, *Leakey's Luck,* is a fine complement to the memoirs. Her close involvement with both the events and personalities she describes makes her book a particular delight. Moreover, her background in prehistory eminently qualifies her as both biographer and critic, and her book constitutes, in fact, a chronicle of studies of early man in East Africa. Although a small amount of fossil human material had been recovered there in the early years of this century, notably "Olduvai Man" by Hans Reck in 1913, it was Leakey's initial work in 1926 which marked the real beginnings of such studies in East Africa.

The controversy which followed so much of Leakey's life began with the report of the human material from Kanam and Kanjera, recovered during the third East African Expedition, in 1931. Leakey contended that the Kanjera skulls and the Kanam mandible represented evidence of an advanced type of human at a very early period in the Pleistocene. This controversy has yet to be finally resolved and his search for a morphologically more modern type of man in the early Pleistocene continued unabated throughout his life. It is ironic that whereas his own candidates for this group, which included *Homo habilis,* have not found wide acceptance in the field, his son Richard's discoveries at east Rudolf have been accepted, almost without equivocation, as very early members of the genus *Homo.*

But Louis Leakey's contributions to the development of Palaeoanthropology as a discipline, far outweigh such "bones of contention", to use Mrs Cole's phrase. In 1947, he organised the First Pan-African Congress on Prehistory, which was held in Nairobi. This Congress represented an important turning point in the evolutionary study of man; not the least of its effects was to remove the focus of fossil man studies from Asia and to re-direct it to Africa where it has remained ever since. At the Congress in 1947 scientists from many different and yet inter-related disciplines met and discussed aspects of their own research involving human evolution in Africa. It was here that Le Gros Clark, in opposition to then widely held opinion, gave convincing arguments why the South African Australopithecines should be included within the human family. It was also at this time that the enormous potential and importance of the East African Miocene deposits became more widely appreciated. As a result of this Congress Leakey received a grant to continue his explorations in deposits of this age and in the following year Mary Leakey found the skull of *Proconsul* on Rusinga Island; to date, this remains the only Miocene hominoid skull known.

Both books are rewarding. *By The Evidence* is a unique and personal anecdotal remembrance, whereas *Leakey's Luck* provides a broad perspective on East African prehistoric studies.

G. E. Kennedy

Spectroscopy from end to end

Molecular Spectroscopy. By Ira N. Levine. Pp. x+491. (Wiley-Interscience: New York and London, June 1975.) £10.50.

Organic Spectroscopy. (A Macmillan Chemistry Text.) By William Kemp. Pp. xvi+248. (Macmillan: London and Basingstoke, June 1975.) £7.95 hard cover; £2.95 paper.

SPECTROSCOPY not only covers a wide frequency range but also has a wide range of interested practitioners from devoted theoreticians to organic chemists. These two books serve opposite ends of this range. Take the area of the ultraviolet spectroscopy of aromatic molecules. An organic chemist, hoping to identify and estimate a small amount of such material in solution before tea-time, would find *Organic Spectroscopy* a valuable reminder, or even introduction, for what is needed. But a PhD student with a research topic to identify the electronic structure and symmetry of an excited electronic state and determine quantitatively its origin, vibrational structure and molecular geometry could usefully study much of *Molecular Spectroscopy* before he starts and all before he faced his examiners.

Both are good books of their kind, although each carries a slight feeling of distilling the best parts of their predecessors rather than adding a new consistent approach. For instance Levine, who is admittedly re-writing parts of, and rearranging, his own earlier book, uses something like a Dirac bra/ket nomenclature for matrix elements but avoids, except for a few equations in chapter 8, the simplicity its full use would bring. He also refers to SI but does not use these units and consequently uses the c.g.s. Gaussian equations rather than rationalised m kg s A versions and associated quantity calculus—as increasingly favoured in schools and essential for consistency with SI. The material seems scientifically sound and reliable and the coverage appropriate, including a new section on photoelectron spectroscopy, although optical rotation and circular dichroism are regrettably absent. I am continually embarrassed by my failure to answer my colleagues who ask "Can you give us a simple picture of what is happening to the molecule and its electrons as optical activity is observed?"

Kemp's book does include this topic briefly, as well as mass spectroscopy. The whole is written in simple language and seems to be appropriate for the bench chemists for whom it is intended, with the slightly more difficult parts identified for digestion at a second reading. The infrared section seems one of the best and I am rather less happy with the one on nuclear magnetic resonance, which attempts to explain sophisticated ideas with a classical picture.

Both are well produced and value for money.

D. H. Whiffen

Intimate knowledge of Wales

The Prehistoric, Roman and Early Mediaeval Field Monuments. By Christopher Houlder. Pp. 207+29 plates. (Faber and Faber: 1975.) £4.50.

FABER'S series of archaeological guides under the editorship of Glyn Daniel grows apace. To the British coverage, already including James Dyer's highly successful *Southern England*, the present volume makes a very welcome addition. It's a surprising fact that this is the first adequate guide to Welsh sites ever to be published.

The author divides the principality into eight regions, determined by the natural structure of the land, and assigns a chapter to each. Within the regional structure all the major sites the listed and briefly described. The text is liberally sprinkled with plans, illustrations of objects and a well chosen series of aerial photographs and includes good references and such tourist information as is necessary. All that, and the accompanying short introductory sections containing information on museums, archaeological organisations, ordnance survey maps, suggested tourist centres and so on, make this an ideal handbook for visitors.

Where the book is a little thin (and this, one suspects, is no fault of the author) is in the actual body of archaeological background information given. Admittedly, there is a seven page outline of the archaeology of Wales which is a masterpiece of intelligent compression, and wherever possible ancillary information is slipped into the main text, but even so it will be difficult for the general user to relate the sites he visits to the development of human societies in the Welsh landscape. The general context would have benefited from a fuller treatment. For the more archaeologically inclined, the bibliography will be helpful and the simple cross referencing scheme will enable the reader quickly to find the principal published account of most sites.

The author is to be congratulated in producing a succinct but invaluable guide to Welsh field archaeology—a distillation of his own intimate knowledge of the landscape and its early inhabitants.

Barry Cunliffe

Erratum. In the Book Reviews Supplement (*Nature*, **258**, November 6, 1975), the reviewer of *The Seas* by Sir Maurice Yonge and Sir Frederick Russell (page 36) was in fact C. E. Lucas. We apologise for any inconvenience or embarrassment this error may have caused.

Nuclear magnetic resonance and biochemistry

Nuclear Magnetic Resonance in Biochemistry: Principles and Applications. By Thomas L. James. Pp. xii+413. (Academic: New York and London, June 1975.) $26.50; £12.70.

IT is now clear that nuclear magnetic resonance (NMR) studies can provide detailed structural, conformational, ionisation and kinetic information about large biological molecules, some of which is inassessible using other techniques. Not surprisingly, many biochemists and biophysicists wish to learn more about the theory and application of the NMR technique. Dr Thomas' book is aimed directly at such readers who will undoubtedly find it useful reading. Starting from first principles, the text describes all the relevant techniques and develops the necessary NMR theory for tackling biological problems. Special emphasis is placed on the theory related to exchange and relaxation effects, which are the most important aspects for biological applications. The theoretical sections are clearly written and, for the most part, the reader should be able to see how the final parameters (such as exchange rates and correlation times) can be calculated from raw data (line widths and relaxation times). In a book of this type, however, it would probably have been useful to work through more examples from the raw data to the final results.

In the section on spin–spin splittings (pages 95–97), a confused definition of the signs of coupling constants is given and it is also incorrectly implied that 1,2-disubstituted ethanes give A_2B_2-type spectra.

An extensive review is given of the applications of NMR to studies of polypeptides, proteins, protein–ligand interactions, membranes and water in biological systems. This section provides an interesting descriptive account of most of the applications to date. Although no attempt is made to evaluate critically the conclusions of the various papers, this part of the book (about 180 pages) constitutes a most useful reference source.

I can recommend the book to anyone interested in learning how to apply NMR to biological problems.

J. Feeney

Dictyostelium discoideum

Dictyostelium discoideum: a Developmental System. By William F. Loomis. Pp. x+214. (Academic: New York and London, May 1975.) $19.50; £9.35.

Dictyostelium discoideum is undoubtedly one of the most intriguing and fascinating organisms that biologists and biochemists have sought to study. It can be cultured as separate amoebal cells showing no apparent cellular interaction and yet at will it can be induced to mass together and develop into a multicellular body that can move and be responsive to light and heat signals in its environment. It is also very good at taking punishment meted out by the curious investigator. It can be squashed, pricked, stained, drugged, decapitated or thoroughly dissociated to separate cells and still resurrect itself and proceed with its planned development. In this book Dr Loomis records and discusses the descriptive and analytical work of many such investigators.

In the early part of the book, the life cycle and various stages of the development are discussed, together with a review of some of the genetic experiments that have been carried out with the organism. After chapters detailing the analysis of cellular enzymes and macromolecular composition, the author then reviews the research concerned with the effects on development of metabolites and such agents as light, heat, cell density, mechanical barriers, dissociation and mutation. The book ends with a discussion of the ubiquitous biological problem of the control of tissue proportions and the various theories that might account for the surprising constancy of ratio of stalk cells to spores in *Dictyostelium*. Such a division into chapters concerned either with developmental stages or selected topics covering several stages must have caused problems for the author over the subdivision of information without causing undue repetition. Generally this difficulty has been skilfully overcome in this book and except for the topic of cell sorting out which is rather awkwardly divided between pages 69–71 and 169–170, the chapter arrangement is rational and helpful to the reader.

The book is written in a style that is lucidly descriptive and unambiguous. The writing lacks flamboyance or sparkle, but whether this is a point for praise or criticism depends on whether one wishes to be succinctly informed or entertained. The illustrations are plentiful and of good quality and include photomicrographs using Nomarski optics and scanning and transmission electron microscopy; they add a great deal to the interest of the book.

The account of the various experiments described in the text is, inevitably, somewhat subjective, although only occasionally is this tendency obtrusive. If any criticism is due, it is possibly to the occasional failure of the author to make it clear whether a particular statement is a considered review of the consensus of opinion or the author's own particular viewpoint.

The author notes in the preface that he has attempted to put into focus most of the major studies on *D. discoideum* and generally I feel that he has been successful. One area of research that might also have been included as a major study is that of the work on radiation effects by Dr R. A. Deering and his colleagues at Pennsylvania State University using normal and γ-ray-resistant strains of the organism. The bibliography which is fairly comprehensive is also lacking in many of the interesting papers from Dr Deering's laboratory.

In general this is an excellent book and I would recommend it to anyone wishing to acquire an overall impression of the past and current areas of research using *Dictyostelium*. Dr Loomis has carefully trodden the narrow path avoiding both superficiality and academic pendantry, and has succeeded in producing a book that is both readable and thought provoking.

P. C. Newell

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Astronomical applications of interferometer

The Intensity Interferometer: Its Application to Astronomy. By R. Hanbury Brown. Pp. xvi+184. (Taylor and Francis: London, November 1974.) £6.

THE original stellar intensity interferometer at Narrabri, New South Wales, finished its program in February 1972, and Professor Harbury Brown has celebrated its achievements and looked forward to the future by writing this account of its construction and use. If only more research programs could be recorded in this way. Reading the book is to acquire twenty years of experience painlessly in a few hours. One can read how physicists proclaimed that the interferometer could not work; that the Australian customs officials were fought for a year and defeated; how the main steel tubes supporting the reflectors sagged; how the reflecting surface was accidentally stripped off; and how parrots pecked through the electric cables. Substitute appropriate personalities and pests and you have a story which could be adapted to describe the progress of many another astronomical project. In many ways the intensity interferometer was vulnerable and exposed to attacks of all sorts, even more so than most telescopes, because of the originality of the design and the rural surroundings. The lessons learned must be appropriate to other ventures.

This is not to say that one is reading a chapter of accidents; far from it, as the difficulties were overcome and most of the book contains a straightforward account of this successful work—for example, one has the full theory of the intensity interferometer and a choice of simpler explanations to suit one's taste. The technical description is given in considerable detail, although without an exact analysis of the system of lenses. The scientific results subsequently described show the power of the new technique. Although the primary program, to determine the angular diameters of stars and their emergent fluxes, was carried out, many other interesting possibilities have arisen from the studies of individual stars and multiple stars. If the reader hasn't already guessed, this leads to a proposal for a new instrument to extend and continue the programs. One is pleased to record that the second interferometer is going ahead and to wish its builders as much success and almost as much excitement.

R. G. Bingham

Cytogenetics

Chromosomes and Cancer. Edited by James German. Pp. xxv+756. (Wiley: London and New York, November 1974.) £17.40.

IT is well established that the great majority of cancer cells have grossly abnormal chromosome complements. Since malignancy is, in essence, a heritable loss of growth control, it is obviously tempting to relate its development to these visible derangements of the genetic material. Because some clearly malignant cells have apparently normal chromosomes, and because the chromosome abnormalities observed seem (with a few notable exceptions) to lack specificity, it has been argued that these microscopic rearrangements are merely a by-product or late result of the malignant process.

The book is the first in a series devoted to cytogenetics, although we are not told what other topics will be covered. It starts rather charmingly with a brief scientific autobiography by Curt Stern, followed by a review (by U. Wolf) of Theodor Boveri's 1914 publication *On the Problem of the Origin of Malignant Tumours* in which the questions of this field were first clearly formulated. The body of the book consists of what are essentially 22 review articles, grouped into four sections: disturbances of the genetic material; cancer as a clone; cytogenetics of certain specific cancers; and special approaches. The chapters stand pretty well independent of one another, with relatively little cross reference. This inevitably leads to some repetition —the classification of visible chromosome damage into gaps, breaks, and so on, is covered by three different authors. There is also some imbalance in the amount of space devoted to different topics—for example, Makino uses 31 pages (excluding references) to review in minute detail the admittedly fascinating but limited subject of canine venereal tumours, which are seemingly transmitted from animal to animal by whole-cell transfer, whereas H. J. Evans covers the entire field of radiation cytogenetics in only five pages more.

As with any multi-author book, styles vary widely. Personally I do not find this a disadvantage. The quality also varies quite considerably, from chapters which are rather dull catalogues of major publications, to some really excellent resumés of complex subjects. I particularly enjoyed David Comings arguing for uninemy—"What is a chromosome break?"—O. J. Miller's section on cell hybridisation, and S. M. Gartler's review of the use of biochemical markers in mosaic individuals (including females heterozygous for X-linked markers) to study the clonal origin of tumours.

The recent and unexpected metamorphosis of the Philadelphia chromosome from a deletion into a translocation caught some authors with their hypotheses showing. In fact, the subject matter of the book by and large antedates the application of chromosome-banding techniques to tumour cytogenetics; and much new information is bound to emerge over the coming years. Dr German writes in his introduction that, for this very reason, it is "a highly suitable time to review previously accumulated knowledge". I thoroughly agree with him. Recent advances must be read in the current literature—a book like this justifies its cost by summarising, in digested and digestable form, a vast and complex literature which will generally be relevant for a good many years to come.

The general standard of contributions is high. I recommend this book to anyone interested in the subject of cancer, either for a quick overview of accumulated cytogenetic knowledge, or as a detailed reference source for those actively engaged in the field.

Martin Bobrow

Defects in solids

Theory of Defects in Solids: Electronic structure of defects in insulators and semiconductors. (Monographs on the Physics and Chemistry of Materials.) By A. M. Stoneham. Pp. xix+955. (Clarendon: Oxford; Oxford: London, May 1975.) £29.25.

THIS is a particularly worthy book, one which has long been needed by theoretician and experimentalist alike. Approximately the first half of the book leads one through a very detailed discussion of all aspects of the necessary theory, stressing the role of the perfect lattice, the electronic structure of the defect, and lattice dynamics. All the major theoretical models are discussed and their shortcomings made clear, in some cases brutally so. I think that the solid-state theorist will enjoy this part of the monograph particularly, although for the beginner it will be a daunting task. Many theorists will also find the second half of the book invaluable as the comparison of experiment and theory is especially well presented. Not only will it be informative, but it may imbue the reader with the motivation, so evident in all Stoneham's work, to attempt calculations of direct applicability to experiments already done and still to be done. This last part of the book will be much used by the experimentalist, not simply "to know what, if anything, they should believe of the present theories" but also because it will give them a clearer vision of the interaction between theory and experiment in solid-state physics.

There are various areas of defect physics which are not treated as they do not fall within the remit of the author to concentrate the electronic properties of defects in insulators and semiconductors. Thus, there is no attempt to treat line and planar defects, nor mechanical properties and defect production mechanisms. If this restricts the readership then the price will do so even more. Such possible restriction is a pity as this is an important book of considerable scholarship.

B. Henderson

Treatise on Solid State Chemistry. Volume 2: Defects in Solids. Edited by N. Bruce Hannay. Pp. xiii+527. (Plenum: New York and London, 1975.) $42.00.

THIS volume is the second in a series of works which aim to present the properties of solids from a chemical viewpoint. Studies of defects, imperfections or impurity ions may be described equally well from a basis of chemistry or physics and this volume has a definite chemical bias but is still suitable for physicists or materials scientists. As the existing literature on defects is predominantly oriented towards physicists it is refreshing to read a book with this change of emphasis. Within the volume seven aspects of defect properties have been selected and each is presented by a different author. Chapter titles range over the following topics: electronic structure and spectra of impurities in the more ionic crystals; colour centres in ionic crystals; dielectric properties; transport properties; semiconductors; magnetic properties; mechanical properties. In a single volume such a range of separate contributions has lead to some differences in the level of presentation but in general the book is suitable for first year postgraduates or people who are unfamiliar with some aspect of solid state chemistry. It is not easy to generalise on the work of seven authors but I felt that the book was clear and informative in those areas with which I am least familiar. In more familiar areas, however, it was apparent that much of the book must have been written several years ago. For an introductory text this is a minor fault but one consequence is that the references tend to be rather old and in an active field such as solid state chemistry this is a pity. Similarly in a few cases defect structures are discussed in terms of outdated models. In spite of these limitations this volume on defects in solids should prove a useful text.●

P. D. Townsend

Principles of Microbe and Cell Cultivation. By S. John Pirt. Pp. x+274. (Blackwell Scientific: Oxford and London; Halstead: New York, 1975.) £10.50.

THIS book has been written for students of microbiology and as a reference book for research workers involved in the problem of microbial cell growth. The treatment of the subject is a clear simple mathematical one which should enable the reader readily to understand the dynamics of the different techniques of cell cultivation.

The book is divided into 25 chapters with an appendix of the symbols and abbreviations used. The initial chapters are devoted to an historical outline and to a consideration of the general parameters of cell growth. Batch and continuous culture methods of cell growth are then discussed. The next four chapters deal with the supply of energy and oxygen to growing cells. The other factors affecting growth, general nutrition, temperature, *p*H and water activity are dealt with in separate chapters. There are a number of chapters of interest to microbiologists involved in the production of materials by microbial processes, for example, product formation, growth lag, and batch cultures with substrate feeds.

The subjects of interest to microbial ecologists are dealt with in chapters on slow growth rates, mixed cultures, and growth on surfaces as microbial films. Finally, growth of colonies on solid media, and mathematical models of biomass autosynthesis are dealt with.

Microbial growth is usually treated as a biochemical phenomenon and many excellent textbooks are available which use this approach. The dynamics of cell growth, however, are usually given only superficial treatment; thus, Professor Pirt's book fills a much needed gap in the microbial literature. But both approaches have their merits and both types of book are needed to obtain a balanced viewpoint.

It would have been useful to widen the discussion a little more to show how the ideas outlined could be applied to specific problems. On several occasions the primary references to the subject are not given.

Overall Professor Pirt's book is a very useful addition to the microbial literature and should be read by all those interested in the dynamics of microbial and cell cultivation.

D. C. Ellwood

The New Mars: Discoveries of Mariner 9. By William K. Hartmann and Odell Raper. Pp. 179. (NASA Office of Space and Science: Washington, DC, 1974.) $8.75.

THIS is in many ways the definitive book to emerge from the Mariner 9 adventure, produced with the cooperation of the Mariner 9 Science Exploration Team. As such it provides a well-written insight into one of the most successful scientific endeavours undertaken by man, and should be of interest to many people outside the circle of specialist astronomers and space scientists. For the price, it is worth having just to look at the lavish illustrations; serious planetary astronomers will not gain much from the text that has not already been published, but they may find the general overview of Mars useful, together with the bibliography provided in an appendix.

John Gribbin

Books brief

Interfacial Electrochemistry: An Experimental Approach. By E. Gileadi, E. Kirowa-Eisner and J. Penciner. Pp. xviii+525. (Addison-Wesley: Reading, Massachusetts and London, June 1975.) Cloth $19.50; paper $13.50.

THIS book is divided into two sections, theoretical and practical. The theoretical section (150 pages) is a brief account of basic interfacial electrochemistry with three chapters covering corrosion, energy conversion and electroplating. The theoretical chapters succeed in the authors' aim of providing the fundamentals of electrode kinetics and double-layer theory; the other three chapters, however, are are inadequate and could well have been omitted.

The rest of the book is an excellent advanced practical text. Fourteen chapters describe current techniques of electrochemistry. The arrangement of the subject matter is good. Starting with the basic electronics (emphasis on op-amps) and reference electrodes, the reader is led through the majority of the important techniques of electrode kinetics and interphase studies applied to well defined systems. There are a few criticisms, ring-disc electrodes are omitted and impedance methods are not given sufficient space; electrometallurgy and electrocrystallisation are not dealt with. But these are of small importance, the main references are there at the end of each chapter and the reader is guided by the Authors to the major works of electrochemistry.

The book is a valuable addition to the literature of electrochemistry, and the price is reasonable by contemporary values.

N. A. Hampson

Normal Values in Clinical Chemistry: a Guide to Statistical Analysis of Laboratory Data. (Clinical and Biochemical Analysis: A Series of Monographs and Textbooks, Vol. 2.) By Horace F. Martin, Benjamin J. Gudzinowicz and Herbert Fanger. Pp. viii+504. (Marcel Dekker: New York, May 1975.) $37.50.

MORE and more pathology laboratory results, especially those of clinical chemistry, are numerical in form. Clinicians are accustomed to interpreting such results with the aid of the normal range, a range of values intended to include those to be expected from a large proportion (conventionally 95%) of healthy individuals. This is clearly a statistical concept, and the statistical problems of estimating the limits of the normal range have received a good deal of attention. In fact these problems are quite straightforward; the real difficulties are logical ones, notably in deciding on what is meant by the "normal" population, and practical ones in obtaining the necessary data.

This book attempts to provide the clinical chemist with the statistical techniques needed to assess normal limits, but the attempt is far from successful. A very large literature, not all of it relevant, is extensively surveyed, but the uncritical approach and lack of logical organisation makes it almost impossible to follow the authors' drift. Explanations of the basic statistical principles vary from obscure to downright misleading. Even the practical problems are quite inadequately handled; it is never quite decided whether or not normal limits can be derived from data relating to hospital patients, and the large effects of the menopause on female biochemistry are not mentioned. Clinical chemists should wait for a better book on the subject, which should be considerably shorter and less expensive.

M. J. R. Healy

[illegible]
SCIENCE COLLEGE
Upper Circular Road
[illegible]

announcements

Awards

The Royal Society has announced the following awards: The **Copley Medal** to **F. H. C. Crick** for his elucidation of the structure and function of DNA and his continuing important contributions to molecular biology; The **Davy Medal** to **T. M. Sugden** for his contributions to physical chemistry including particularly the analysis of the reactions occurring in flames; The **Hughes Medal** to **R. H. Dalitz** for his contributions to the theory of the basic particles of matter; The **Leverhulme Medal** to **F. L. Rose** for his contributions to the application of chemical science to industry.

International meetings

January 5–6, **Aspects of water use,** Loughborough (John Pickford, University of Technology, Loughborough, Leicestershire, UK).

January 5–7, **Solid State Physics Conference,** Manchester (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

January 5–9, **Chemistry and biology of peptides,** California (Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).

January 6, **Photorespiration,** London (Dr B. Halliwell, Biochemistry Department, King's College, The Strand, London WC2, UK).

January 6–9, **Research and medical practice,** London (The Ciba Foundation, 41 Portland Place, London W1N 4BN, UK).

January 12–16, **Minicomputers/microprocessors and their applications to real world problems,** Zurich (Industrial Liaison Office, Room 39–615, MIT, Cambridge, Massachusetts 02139, USA).

January 12–16, **Fermentation technology,** Zurich (Industrial Liaison Office, Room 36–615, MIT, Cambridge, Massachusetts 02139, USA).

January 12–16, **Proteolysis and physiological regulation, and cancer enzymology,** Miami (Miami Winter Symposia, PO Box 520906, Florida 33152, USA).

January 21–22, **Energy–brake or break,** London (Institute of Fuel, 18 Devonshire Street, Portland Place, London W1N 2AU, UK).

January 22, **Volcanic processes in ore genesis,** London (Dr I. L. Gibson, Volcanic Studies Group, Bedford College, London NW1, UK).

January 26–30, **Biology of aging,** California (Gordon Research Conferences, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).

Miscellaneous

Data on Cytogenetic Registers. The International Advisory Committee on Cytogenetic Registers to the Standing Committee on Standardisation in Human Cytogenetics is compiling, updating and publishing a list of cytogenetic registers, together with summaries of aims, objectives and key elements. The Committee has developed a form for the collection of such data and invites individuals who operate Cytogenetic Registers (either in isolation or as part of a larger register system) to write to Dr. James R. Miller, Department of Medical Genetics, The University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

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nature

November 27, 1975

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The Chinese dimension

A MOST remarkable piece of Chinese science is reported on page 286. It seems that as a result of a major redirection in scientific goals during the Cultural Revolution, the Chinese have been able not only to start issuing credible predictions of earthquakes, but also to save lives by firm action. Such success is bound to raise questions about what lessons we could learn from the Chinese, and whether their very distinctive brand of 'science for the people' could with profit be adopted in other countries.

In many ways the Chinese earthquake programme is a unique one. It has been possible to involve amateurs in the reporting process, even to the extent of asking peasants to pass on news of unusual animal behaviour. It has also been possible to rely on an orderly public response to a warning—something very difficult to envisage in southern California. There is something akin to the feeling of a popular crusade in the whole operation; this is hardly a surprise since in the past hundred years at least 200,000 Chinese have been killed in earthquakes (a hundred times the number of American deaths from that cause). But many of those who return from China express considerable concern that the short term advantages of people-involvement will be rapidly squandered if there is no long term policy of upgrading the role of the scholar and scholarship.

Some scholarly facilities are highly praised—particularly libraries—but there are considerable doubts about the adequacy of postgraduate training; indeed in many cases it is almost non-existent. After a three-and-a-half year undergraduate career, the best that the intelligent student can hope for is to be seconded to some field unit where the problems are intellectually demanding and where, at the least, he may pick up some form of scholarship from an older professor with experience of the former style of graduate education. As a means of mobilising a large technical army and convincing the general public that science is working for them, the present method cannot be faulted. It does, however, pose serious problems when it comes to capitalising on genuinely outstanding talents, and there is some doubt that, amidst the welter of activity on every conceivable front, enough criticism goes into the planning of future work.

The American committee, whose report is described on page 286, writes "the local scientific workers described the experiments in which they are engaged. Discussing their work in this manner is apparently an unusual experience for many of these young scientists, and when we questioned or disagreed with them they seemed unaccustomed to the give-and-take of scientific criticism which is so characteristic of the American scientific establishment . . ." As a result, it seems that the Chinese are ever-enthusiastic to measure anything and to try any longshot, regardless of whether or not there is any sound scientific justification for it. In some cases this could pay off handsomely (it is possible that in monitoring animal behaviour the Chinese could be on to something big), but the pay-off is more likely to be in the West, which is better intellectually and organisationally equipped to take anomalous observations, build, refine and use models. It is to be hoped that delegations travelling out of China have picked up some ideas, too, on the running of science.

One of the intriguing questions that arises from the several tens of exchanges of scholarly delegations in the past two years is whether the obvious interest in China is going to be at the expense of exchanges with the Soviet Union. This is partly a political question, of course, and depends on the future of the triangular relationship between Peking, Moscow and Washington. There is, however, little doubt that if the decision were left to the scientists, many would like to cultivate their Chinese contacts, even though the many close friendships that westerners have with individual Soviet scientists would probably not be paralleled in the more anonymous Chinese situation.

There are perhaps two non-scientific reasons why China is appealing—it has an extraordinarily rich cultural scene, and there is an apparent lack of the endless feeling of being watched which has made so many westerners almost relieved to get out of the Soviet Union. But what excites visitors scientifically is the feeling that Chinese science is involved in a great experiment in mutual help; those who do not have their minds ideologically closed want, in some fashion, to see the outcome of the experiment and even to help participate in it. □

Primitive wheat in Afghanistan, showing variability (FAO photo by E. Bennett)

Future in the past

Modern agriculture threatens to destroy the very means by which it developed. The recent establishment of the International Board for Plant Genetic Resources will help to coordinate international efforts to conserve the rapidly diminishing genetic resource represented by traditional varieties of crops grown for generations. Eleanor Lawrence looks at some of the projects now under way.

MOST of the staple crops grown today were first brought under cultivation thousands of years ago. Cultivated plants have spread out from their original centres of cultivation until they can be found today growing in conditions very different from those in which they originated. In the process they have diversified into innumerable genetically different local varieties, each showing some adaptation to the climate and general environment in which they now flourish.

This reservoir of genetic raw material has in the past been of inestimable value to the plant breeder. Over thousands of years of evolution and selection, genes for resistance to many threatening and economically damaging pests and diseases have arisen. Useful qualities such as cold and drought tolerance have developed in varieties growing at the limits of their range. Plant breeders have constantly returned to the older strains for useful genes which can often be found only in a very few varieties or in their wild relatives. Now, however, this invaluable genetic resource is fast disappearing as a result of replacement of traditional crops by the generally more productive newer varieties.

The small fields of primitive agriculture bear little resemblance to the monocultures of industrialised developed countries. Several varieties of the same crop are often grown together, and the individual plants differ greatly from each other. The wild relatives of the cultivated plant may well be present either as weeds in the field or growing along pathways and field margins.

This variability sometimes results in relatively low yields. Indeed, peasant farmers have often selected for reliability rather than high performance, on the basis that in a bad year a small but dependable yield is better than no yield at all. But it is the genetic mixture itself which makes possible the emergence of desirable new forms by fortuitous crossbreeding. These forms may then be selected simply by their superior ability to cope with local conditions, or directly by the farmer.

Agriculture in the developed countries, where intensive plant breeding has been practised, now depends on a comparatively few varieties of the major crops. When grown with modern aids, such as fertilisers, pesticides and herbicides, these produce yields far greater than those possible with the older highly diverse 'landraces'. The gene pool represented by these highly bred crops, however, is restricted by comparison with that represented by the older varieties. The widespread introduction of new strains into hitherto undisturbed areas of genetic diversity, such as the massive introductions of the Mexican-bred semi-dwarf wheats into India and the Near East, actually poses a grave threat to the continued existence of many primitive strains, which are now found only sporadically and in remoter regions.

Where the introduced crops mean higher yields and a better standard of life for the farmer, the disappearance of the older varieties from the fields is inevitable. But the potentially valuable genes which they contain must be preserved, for they will certainly be needed in future crop breeding programmes. Nobody yet knows the full extent of variation available in traditional crops, and many qualities which could be overlooked now might be the very ones desperately needed in the future.

Although the disappearance of traditional varieties is unlikely to slow down, for many crops a reasonably representative sample of the variation in any population can be conserved easily and economically as seed. Seeds of many species remain viable for years if they are kept dry and cold. The optimum conditions for storing many types of seed are now well-known, and gene banks for most important crops have been established in centres all over the world. The old varieties and their wild relatives have been a fruitful source of genes resistance for pests and disease; moreover, they will always have an important part to play in the never-ending race to breed new resistant varieties before the pests themselves progress.

Unusual cultural characteristics sometimes found in primitive varieties have had a revolutionary effect on plant breeding. The semi-dwarf habit in rice, which formed one of the basic traits of the rices of the Green Revolution, was introduced from a primitive variety found in Taiwan. Scientists at the International Rice Research Institute in the Philippines, where the first dwarf rices were bred, are now attempting to combine high yield with the 'floating' characteristic of rices grown for generations in the deep water rice areas around the great rivers of India and South-east Asia.

International efforts to conserve the rapidly vanishing reservoir of genetic diversity began some 15 years ago when the Food and Agriculture Organisation of the United Nations (FAO) convened a technical meeting on plant introduction and exploration. In 1963 an FAO panel of experts on plant exploration and introduction was established, and in 1968 the Crop

Ecology and Genetic Resources Unit, a special section within the FAO, was set up to act as a clearing house for information on genetic resources and to administer the FAO's programme.

The interest this aroused confirmed that the problem was as grave as had been feared. Moreover, the efforts already underway by individual institutes and the FAO itself were only able to tackle the most urgent problems. A relatively large amount of material has now been collected from the more accessible areas, but remoter regions of the world, which contain much of the remaining genetic diversity in many major crops, remain largely unexplored.

A plan for a worldwide network of genetic resource centres incorporating existing institutes and projects within an international collaborative programme was therefore drawn up at a meeting in 1972 sponsored by three bodies: the FAO, the Consultative Group on International Agricultural Research (CGIAR) and the Technical Advisory Committee to the CGIAR.

The programme is coordinated by a committee created 18 months ago, the International Board for Plant Genetic Resources (IBPGR) This falls under the aegis of CGIAR and has a secretariat in the Crop Ecology and Genetic Resources Unit of the FAO. Its budget in 1975 was some $730,000, and a budget of $1.3 million has been proposed for 1976. A year of fact-finding has resulted in several projects now being undertaken with IBPGR funds.

One of the most important projects from the point of view of coordination and information exchange is the genetic resources communication, information and documentation system (CIDS) project based at the University of Colorado at Boulder. This project is intended to realise the full potential of the collections made, which means they must not only be described botanically, but in such a way that the plant breeder can easily identify material which might be useful in a breeding programme. Botanists and plant breeders who wish to use the materials must know details of the original habitat, morphology and local conditions, which can only be recorded at the time of collection. Information on disease resistance and physiological properties can be added later after proper evaluation.

The CIDS project was originally set up, and is still partly supported, by the Crop Ecology and Genetic Resources Unit of the FAO, with the aim of devising a computer system suitable for recording and retrieving information on crop plant genetic resources. The IBPGR has given its support, allocating $237,000 to the project in 1975. It hopes to provide another $360,000 in 1976.

Through visits to existing collections and intensive collaboration with both collectors and plant breeders, the CIDS team has initiated a transfer of genetic resources records into a storable form through a system (TAXIR/EXIR) which is adaptable to any of the principal types of computer with an adequate memory bank. It is hoped that eventually the inventories of all major collections will be filed on this or some other compatible system, which will allow the user to retrieve the collections from the area or with the particular characteristics he wants.

As for the future, some of the field projects which the IBPGR is to finance out of its own fund for 1976 include the Near-East Regional Programme, a programme for the Mediterranean area centred on the Germplasm Laboratory at Bari in Italy, and the development of a regional programme for south and south-east Asia. The Board is also financing collections of rice by the International Rice Research Institute (IRRI) in Bangladesh, Indonesia and other south-east Asian countries.

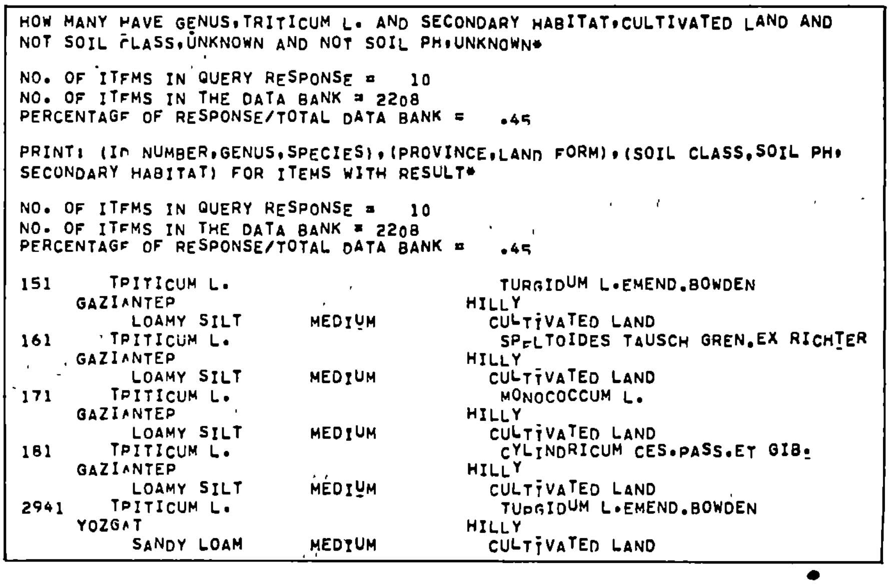
Many important tropical forage legumes originate in Latin America—the pastures which support the Australian livestock industry are made up almost entirely of introduced species, many from Latin America—and the Board has also provided funds to begin a three-year programme of collection, conservation and evaluation of tropical forage legumes and grasses there. This work is to be carried out by the Centro Internacional de Agricultura Tropical (CIAT) in Colombia.

Attempts to improve the natural pastures in Latin America have used native legumes such as *Stylosanthes*, *Centrosema*, *Desmodium* and *Macroptilium* which make important contributions to the nitrogen fixing ability of the pasture and its nutritional value. But the improvement has been at best sporadic, and the CIAT programme aims to make a systematic and representative collection of these tropical forage legumes and their accompanying symbiotic bacteria, the *Rhizobia*, as well as some tropical grasses.

Ethiopia is an immensely important region of genetic diversity under stress and is an especially important area of variation in wheat and barley. Although several collecting expeditions have been mounted over the years, by the Germplasm Laboratory at Bari for instance, the region warrants its own regional genetic resources centre, like that serving the Near-East at Izmir in Turkey. The Federal Republic of Germany offered to finance such a centre several years ago, and the first funds are to be spent in 1976.

Natural disasters as well as human activity can threaten genetic resources. An expedition organised by the French Organisation for Scientific Research Overseas (ORSTROM), financed by the United Nations Environment Programme, is now in the Sahel collecting as much as possible of the remaining native varieties of sorghum and millet. The indigenous varieties are particularly resistant to drought, a characteristic not found elsewhere. After a disaster, restocking tends to be carried out with easily available varieties brought in from outside which lack the drought resistant characteristics peculiar to the indigenous varieties. If the native genetic material can be collected and stored, however, it can be used in future breeding programmes and even be reintroduced if such a disaster strikes again.

With so much to be done, the limited funds of the IBPGR are to be used mainly as 'seed' money to start off some urgently needed programmes. Its other task is to identify neglected areas and encourage potential donors to put their money into the most worthwhile projects—a task which will ultimately prove to be equally important.



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        LOAMY SILT      MEDIUM            CULTIVATED LAND
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    YOZGAT                              HILLY
        SANDY LOAM      MEDIUM            CULTIVATED LAND
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Information retrieved from genetic resources data bank

Eco-business

THOMAS H. JUKES

ONCE upon a mountain there was a man named John Muir. He lived on breadcrusts and tea, and he slept in a blanket beneath the shelter of stunted trees (*Pinus albicaulis*) at timberline. He became famous because he awakened the minds of many people to the need for preserving and protecting the wild meadows, flowers, forests, streams and lakes of the Sierra Nevada and other wildernesses.

But, incredibly, Muir's crusade to save the wilderness gave birth to an organization that became a replica of Big Business. Other groups followed suit. The metamorphosis was an evolutionary process; success attracts money, money employs people, and then the payroll must be met, and, above all, the organization must grow. With fascination, I have watched the change in John Muir's legacy—the Sierra Club. He used to hike with the Club on their annual summer outing to the mountains that started in 1901 and has continued through the years.

For the first fifty years, the outings were on a modest scale. In 1957, the cost of the 28 scheduled trips totalled $1,571, an average of $4 per person per day; and obviously cheaper than staying at home. Then came the boom. In 1972, there were 208 trips, and some cost as much as $1,725 *each* for five weeks, not including jet plane fares. Apparently the Sierra Club has not reached its Limits to Growth.

With growth comes financial responsibility. Those who are engaged in saving the world need money, but they are too busy with important matters to concern themselves with investment. Professional counsellors handle such nagging details. Fortunately, there is a Freedom of Information act that allows such matters to be uncovered. The National Audubon Society, once a birdwatchers' club, has a handsome and diverse portfolio of securities including Strathmore Paper Co., Inland Steel, General Electric, Atlantic Richfield Oil, Walt Disney Productions, Texaco, and Columbia Gas. Moreover, Audubon, the rescuer of birds from slicks and spillage made by the polluting oil companies, has a nice gas and oil business in its Rainey Wildlife Sanctuary in Louisiana which brings in $300,000 annually.

Great huffing and puffing followed these disclosures. The President of Audubon, who used to be Secretary of the Army, said that the Society needed experience in actually producing gas and oil. A police court magistrate, I suppose, should commit a few burglaries to get the feel of the thing. The Sierra Club Foundation is long on Exxon, General Motors, Tenneco, Public Service of Colorado (nuclear reactors), strip-mining firms and pulp-mill companies. The Environmental Defence Fund defended its fiscal environment in 1973 by cashing dividend cheques from Mallinckrodt Chemical, Avon Products, Exxon, and Philip Morris.

Some security analysts say that the relationship of conservationists to petroleum companies is symbiotic, citing the case of the Alaskan Pipeline. The construction of this was delayed because of a campaign, spearheaded by the Sierra Club, after a large stock of steel pipe had been purchased in Japan and delivered to the frozen tundra before the current inflation. The price of crude oil zoomed up during the delay, and now the oil companies are using the low-cost pipe, which will transport oil whose net worth multiplied *in situ* while conservationists fought valiantly on behalf of the caribou . . .

I am told that caribous have developed a habit of sleeping on the wooden drilling platforms in Alaska, apparently to ward off the chill of the permafrost. Oh, well!—John Muir rolled himself in a blanket. May as well keep warm! ☐

international news

A thought for food

by Colin Norman, Washington

To help avert "mass hunger for future decades", there should be an immediate, massive infusion of funds into research on food production and a major overhaul of the agricultural research system in the United States, according to two reports submitted last week to President Ford by the National Academy of Sciences. The reports outline preliminary findings of a two-year study, due to be completed in June 1977, of how research in the United States can help combat the grim prospect of increasing hunger and starvation throughout the world.

Specifically requested by President Ford in response to the World Food Conference, which was convened in Rome a year ago, the reports are surprisingly critical of agricultural research policies in the United States. Although there have been spectacular increases in domestic agricultural production during the past three or four decades—to the point, in fact, where North America is now virtually the world's breadbasket—the reports suggest that research efforts lack central coordination, receive insufficient funds, and pay too little attention to basic studies. The preliminary reports, which are designed to influence the Ford Administration's budget for next year (which is now being prepared), thus offer a prescription for improving the research system, and suggest a number of specific projects requiring an immediate injection of funds.

One of the reports, prepared by a committee headed by Dr Harrison Brown of California Institute of Technology (*World Food and Nutrition Study: interim report*) presents an overall view of how agricultural research in the United States can assist in coping with the world food problem. It draws heavily on the findings and recommendations of the second report, concerned mostly with domestic agricultural research policy (*World Food and Nutrition Study: Enhancement of Food Production in the United States*), which was prepared by a committee headed by Dr Sylvan Wittwer of Michigan State University.

Although it is tempting to suggest that the world food problem could virtually be solved by better distribution of food supplies, the Brown committee points out that, with world population increasing by about 2.5% a year, better food distribution will offer at best a stop-gap solution. A few summary facts speak for themselves.

Developing countries increased their food production by about 2.8% a year during the 1950s and 1960s, an impressive achievement which reflects both increasing yield from individual farms and increasing acreage under cultivation. But their effective demand for food in the 1960s increased even more sharply, by about 3.5% a year, and it will continue to do so for several decades. The result has been a rapid escalation in food imports by the developing countries—a trend which, according to estimates of the Food and Agriculture Organisation, will result in total cereal imports of 100 million tons by 1985.

The Brown committee therefore argues that "the key to adequate feeding of peoples in the developing countries over the coming decades is a sustained trend of very substantial increases in the yield of their farms". The increases, in fact, will have to be even more spectacular than those brought about by the recent introduction of high-yielding varieties of wheat and rice—the so called Green Revolution—and the committee also notes that "there is a parallel requirement for continuing rapid expansion of US yields on our principal export crops".

Such achievements will require "a greatly strengthened research base", the committee argues, and its recommendations essentially boil down to three requirements: increased participation by research institutions and scientists from the United States in international research and development activities; increased attention to several lines of research in the United States which have worldwide implications; and some overdue reforms in the planning and execution of domestic agricultural policy.

● Proceeding from the observation that "it is not feasible in the next decade or two to meet the developing world's massive requirements for research capabilities and research results one country at a time", the committee urges "redoubled US efforts" to help build collaborative international research networks—such as CIMMYT, the International Centre for the Improvement of Maize and Wheat, where Norman Borlaug developed his high-yield wheat varieties. In addition to increased funding for such networks, the committee also urges greater participation by universities and other institutions within the United States in the work of the international research centres.

● As for research projects which merit increased attention, the Brown committee notes that the "prevailing bias" among politicians and research administrators is toward applied research likely to yield quick results. The Wittwer committee is less charitable: "Fundamental research undergirding food production technology has languished for two decades", it asserts. "Because of crop surpluses, political pressures from commodity groups, budgetary reductions, and emphasis on immediately applicable results, a formerly substantial basic research effort . . . virtually disappeared".

The Wittwer committee has consequently drawn up a list of research efforts which have been relatively neglected in the rush for quick results. An expanded research programme on photosynthesis is "urgently needed", the committee suggests. It recommends that federal support of such activities be doubled, from $10 to $20 million a year, and that two or three research institutes be established to spearhead the effort. Similarly, research on nitrogen fixation should receive a large injection of federal funds. "The current funding level of less than $5 million from all sources in this nation for research on biological nitrogen fixation is grossly inadequate", the committee states, and suggests that an immediate escalation to $25 million is needed, followed by a 25% annual increase for the next five years.

Basic genetic research to improve plant yields—including DNA recombination techniques—should also receive a five-fold increase in the present funding level of about $500,000 a year, the committee urges. Other areas singled out for increased attention are the adaptation of chemical fertilizers for use in tropical regions, improved methods of pest control, ways to combat livestock diseases, and a better assessment of land and water resources in the United States.

The Brown committee accepts the need for increased efforts on those projects, notes that "there is little to be gained from identifying research work that needs acceleration unless US Government food research budgets are increased sharply", and recommends that expenditures on agricultural research should be increased by at least 50% over the next two or three years.

● Agricultural research in the United States is funded chiefly by block grants to a conglomeration of many federal, state and private research institutions, and both the Brown and Wittwer committees suggest that it lacks central coordination and direction. It is not the first time that committees of the National Academy of Sciences have criticized the system. Three years ago, in one of the most candid assessments of a federal research effort ever to emerge from the academy, a committee under the chairmanship of Dr Glenn Pound of the University of Wisconsin argued that agricultural research supported by the Department of Agriculture is often pedestrian, badly managed and inefficient.

The Wittwer committee seems to have accepted the thrust of that assessment. It recommends that a top-level National Agricultural Research policy Committee should be established to develop a national research policy. Moreover, the committee states that "many agricultural research administrators are of the opinion that (the Department of Agriculture) has not been an effective proponent of agricultural research, that it does not now provide for adequate consideration of the problems and needs of research in its top-level deliberations, and that it lacks administrative and budgeting arrangements that can effectively guide research in response to national and regional needs". It therefore suggests a number of administrative changes within the Department of Agriculture designed to coordinate the activities of its fifteen research divisions, and to enhance the status of agricultural research.

As for funding mechanisms, the committee urges that diverse sources of support for agricultural research be brought together. It also suggests that more funds should be available for support of individual research projects, assessed by peer-review panels and supported in the same manner that biomedical research is supported by the National Institutes of Health.

Although some of those suggestions were incorporated into the report of the Brown committee, it promised to give more thought in its final report to the manner in which overall food and nutrition policy should be developed.

Finally, it should be noted that the Brown committee clearly acknowledges that, in dealing only with the limited topic of agricultural research, it necessarily neglects some of the prime considerations in the world food problem, such as population, education and economic planning. Moreover, the committee also states that it does not "assume superior US wisdom on how to achieve development purposes, but only a capability and willingness to handle research problems and to collaborate with others in problem solving where our participation is welcomed". □

Pesticide perspective

ANOTHER shot was fired last week in the long-raging battle between two of the main schools of thought on the balance to be struck between solutions to the world food problem and their environmental consequences.

Dr Cleve Goring, the director of research and development in plant science for the American-based conglomerate Dow Chemical, nailed his colours firmly to the mast when he spoke at the British Insecticide and Fungicide Conference, held in Brighton.

The three-fold increase in world food production needed by the end of the century to feed the world, he said, would probably require a five-fold increase in the use of pesticides. But he warned that pesticides would, along with other agricultural aids, only help to buy time, rather than solve the long-term food problem.

Dr Goring further warned that "extremists" in the "environmental movement and medical research establishment", especially in the United States, would cause the "unjustifiable elimination" of many products now in use from which the future demand for pesticides would primarily be met. They had to "develop some perspective on the largely imaginary horror of a 'Silent Spring' and the minute amounts of pesticide chemicals that occur in our food".

In speaking out so strongly, Dr Goring drew attention to the apparently increasing plight of even the largest chemical companies engaged in pesticide production. There is evidence that work on new pesticides is being cut not simply as a result of the effects of the recession on research and development but also because the returns are too low for other reasons.

It is accepted even by some environmentalists that the balance is wrong between the time it now takes to develop a new product and the period that the patent laws last. Moreover, it has now become apparent that the new products least likely to yield an economic return are actually the safest in environmental terms because they are also the most selective in their effects.

Dr Goring referred to these matters when he outlined four problems of special concern in the development and use of pesticides:

- The excessive time required in some countries to bring a product from the discovery stage to the market place.
- The need for a "benchmark system" to evaluate the environmental acceptability of new pesticides.
- The lack of agreement between countries on uniform test procedures and tolerances.
- The lack of a workable policy for products reputed to be carcinogenic.

On the increasingly difficult problem of carcinogenicity, Dr Goring had a sharp word for the US Environmental Protection Agency (EPA). He described its recent list of cancer principles as "perhaps the most serious problem", and pointed out that a published list of suspected carcinogens included "many of the natural materials essential to living systems".

But given Dow Chemical's place in the market—sales of pesticides and consumer products last year accounted for more than 10% of its total sales of almost $5,000 million, making it the eighth largest chemical company in the world—Dr Goring's concern was naturally with what he called "over-regulation" of pesticides, which had advanced "beyond all reason".

He was reflecting the view of those who believe that the chances of further chemical breakthroughs were now being reduced. But this view seems to be about as widespread as the environmentalists' belief that there are enough pesticides in existence already. Between the two, however, is an even more widely argued contention that it is not so much the pesticides themselves which are dangerous as the manner in which they are used. As one expert put it last week, "people need not be against their use, only against dangerous substances carelessly used".

Nuclear deals proliferate

THE path towards nuclear non-proliferation is tortuous and often contradictory. This was revealed again recently when more nuclear deals involving less developed countries were announced at about the same time as further meetings of the Nuclear Supplies Group took place in London.

The group, whose members include most of the world's nuclear countries–the USA, the USSR, the UK, France, West Germany, Canada and Japan—wishes to prevent the use of fissile waste products for the manufacture of nuclear weapons. But some of its members are also solidly encouraging the use of nuclear power outside their own privileged circle.

In recent months deals have been reported between France and South Korea for a plutonium separation plant, and between West Germany and Brazil for complete nuclear systems including fuel reprocessing. Secret dealings between West Germany and South Africa have also been brought to light.

Now the UK has joined at least six other countries, including Australia and South Africa, which are interested in Iran's ambitious nuclear programme, and has announced a dual agreement that offers both training and technical assistance, but no reactors.

And the United States apparently promised Egypt two nuclear power reactors, for use in desert desalination plants, during President Sadat's visit to Washington earlier this month. At the same time, the influential Cairo newspaper *Al Ahram* reported that it was Egypt's intention to build 10 nuclear power stations during the next 20 years.

Large as it is, the Egyptian programme barely holds a proverbial candle to that planned in Iran, where the general aim is to make nuclear power the prime source of energy in the country by the 1990s. This will involve some 20 reactors with a combined capacity of around 23,000 MW—more than double Egypt's proposed 10,000 MW, which is its target for the year 2000.

Fears that Iran may be tempted to establish itself as a full nuclear power have not yet manifested themselves as they have over Egypt's plans, in spite of the stimulus that India's nuclear explosion obviously gave to the Shah. The Middle East situation, where Israel's nuclear potential has increased, looms larger in the diplomatic mind, as Dr Kissinger implied when he quickly confirmed that an acceptable arrangement to safeguard the security of the by-products had been worked out with Egypt.

The issue is clearly important to the Nuclear Suppliers Group. The British Foreign Secretary, Mr James Callaghan, emphasised the matter at the UN in September, pronouncing that "the statesmen of the world have a moral duty to act before it is too late to work out the means of controlling the nuclear threat". The talks in London, in fact, were apparently convened in order to discuss the proposals he had made in the General Assembly.

In the meantime, however, the nuclear countries continue to deal among themselves. The British Nuclear Power Company has signed an "enabling agreement" with the Soviet Union on the exchange of experience, techniques and possibly materials connected with the so-called 'steamer' reactors due to come into operation in the UK in the 1980s but already working there. And the efforts to bring Canada's successful technology to the UK continue.

There is evidence of increasing resistance within the nuclear countries to the elaborate nuclear power programmes at home But so far nothing similar has revealed itself in countries wanting such programmes. Only the Nuclear Suppliers Group is showing signs of concern. But it is only the Nuclear Suppliers Group that is acting. to satisfy those wants. □

Round Britain

TEN countries agreed last week on a multi-million pound international programme of five research projects, all based in the UK, designed to develop new technology in the use of coal and so help to solve the energy problem.

The biggest project, costing £10 million, involves the design, construction and operation of an advanced experimental plant near Barnsley which will apply a new technique to coal. It will be financed by the UK, the USA and West Germany.

The technique, known as fluidised combustion because powerful air jets turn burning coal into a bed of red-hot ash that looks and acts like boiling liquid, is said to offer important advantages over conventional steam-raising plant.

Its compactness means a lower capital cost—a big advantage in an era of economic stringency. In addition, it has a high thermal efficiency even with low quality coal, and it cuts pollution by retaining sulphur in the ash bed. It is these advantages which the project is designed to assess.

The other four projects, which cover research services, will together cost about £1 million a year, to be financed by the 10 countries—Austria, Belgium, West Germany, Italy, the Netherlands, Spain, Sweden, Turkey, the UK and the USA

These aim to estimate the reserves of economically recoverable coal, provide information on coal as an energy resource and assess the costs of new techniques for mining and using coal. A mining technology service will also be established.

The programme is the result of the efforts of the coal technology working group, operating under the aegis of the International Energy Agency. The group is headed by Mr Leslie Grainger, the science member on the UK's National Coal Board, which will be the operating agent for each of the five projects. The hope is that the programme will cut the costs of heat for industry and for generating electricity by making a more efficient use of coal.

● An agreement concluded earlier this month will effectively make the UK's Chemical Society owners in the British Isles of all the abstract data produced by the American Chemical Society's Chemical Abstracts Service (CAS) In exchange, the CAS will receive payment in kind in the shape of the specialised expertise developed by the UK Chemical Information Service (UKCIS), a part of the Chemical Society based at the University of Nottingham.

Chemical abstract services are quite big business nowadays, with a total of 5 million chemical compounds known at present and 5,000 new ones being discovered every week. Chemists report their work in fully 14,000 journals.

Even keeping up with the abstracts is itself a considerable chore for many chemists, and the cynics say that it is sometimes quicker to do a simple experiment (to determine a melting point, for example) than to look up the information in *Chemical Abstracts* (the present five-year index of which runs to 68 volumes!).

Although the CAS spends some $25 million a year putting the abstracting material on to a computer and producing *Chemical Abstracts,* it has paid little attention to the problem of how to manipulate further such a vast data base. In the USA, information centres run by bodies like Battelle and Lockheed reprocess the information for their own particular purposes.

The UKCIS, however, has made a point of developing personalised services by making available to individual chemists a current-awareness service which caters for their individual interests. It also, for example, publishes subject-oriented bulletins, 'Macroprofiles'.

At present the UKCIS markets and distributes *Chemical Abstracts,* and some other publications and services of the CAS, in the British Isles. As its contribution to the CAS it also produces the 5% or so of all CAS abstracts and index entries which originate in papers published in the UK.

The annual budget of the UKCIS is, however, small beer by comparison with its American counterpart. Its non-capital expenditure at present is £1.2 million a year. But, like the CAS, its aim is to break even.

The UKCIS can obviously widen its horizons quite considerably now that it is much more than simply an agent for the CAS. In particular, it is actively considering, together with the Department of the Environment, the setting up of a data bank on environmentally significant chemicals Called DESCNET, it would allow biological data on chemical compounds to be stored with other information about them and "should assist in the freer flow of information concerning potential environmental hazards".

● The Post Office exhibited much of its current work in telecommunications research and development last week when the Queen opened the new £11 millions Post Office Research Centre at Martlesham Heath in Suffolk. But most of it was unexpectedly overshadowed by the news that the Post Office is to place major contracts with the telecommunications industry for the development of a computer-controlled switching system code-named System X.

The new system, which will use the latest microelectronics technology, will lead to a modern telecommunications network in Britain capable of handling the wide range of services expected to be in demand by the 1980s, including visual transmission and high speed data print-outs. The development of System X, which is expected to take from five to seven years and cost up to £100 millions, is reportedly the biggest single item of expenditure of the Post Office's annual research bill.

The joint development programme will involve about a dozen contracts with such firms as Plessey, GEC, STC and Pye TMC, the final details of which have yet to be drafted. Advanced as System X is compared to existing telephone switching systems, it is unlikely to supercede current Post Office research work in this field. Both the new waveguide system, which is capable of handling up to half-a-million transmissions simultaneously, and developments in the field of fibre optics will provide important contributions to it. Indeed, it is because System X is based on a series of sub-systems that it offers the Post Office a good degree of flexibility as the development matures.

● A research team at the Harwell Atomic Energy Research Establishment has developed a miniature, nuclear-powered battery which may, quite literally, provide a boost to sufferers with certain types of heart disease. Designed to fit into a small "pacemaker", the new battery works on the thermocouple principle, and because the power output of its plutonium 238 heat source falls off by only about 1% a year it could have an implanted lifetime of 10 or even 20 years. By contrast, conventional chemical pacemaker batteries rarely operate for more than three years before they need to be replaced surgically. The Department of Health and Social Security has ordered 100 pacemakers from British Nuclear Fuels Limited (BNFL) for chemical trials, and the device, now costing about £2,000, could be worth about £10 millions to BNFL

correspondence

Food for thought

SIR,—Dr Magnus Pyke in his article "All the food that's fit to eat" (October 23) notes that the "Society of Public Analysts, now the Society for Analytical Chemistry", was established in the same year as the Sale of Food and Drugs Act 1875. In fact, the first president and council of the new society were elected in December 1874, and were instrumental in influencing certain amendments to the 1875 Act, which was first introduced to the House in February 1875. The society was known as the Society for Analytical Chemistry from 1954 to December 1974, and from January 1975 has continued its activities as the Analytical Division of the Chemical Society.

Yours faithfully,
F. D. GRIMMER

The Chemical Society,
London W1, UK

Aiding Vietnam

SIR,—In his report "Vietnamese Journey" (November 6) Arthur Galston put forward various suggestions for an American aid programme to help Vietnamese scientists. These included sending scientific literature, equipment and visiting scientists to Vietnam.

On the British scene two of these recommendations have been in operation for several years. The Medical Aid Committee for Vietnam has sent considerable quantities of current medical literature to doctors in Vietnam since 1967, and in 1973 began sending scientific literature to Professor Hieu. Many British scientific institutes have assisted us in this work by making donations, including the Biochemical Society, the Institute of Physics, the Royal Statistical Society and the Nutrition Society. Similarly many libraries, including the Library Association, now contact us when they have material which is surplus to their requirements and which we consider suitable for dispatch. All literature is sent free of charge, courtesy of the Soviet Red Cross.

Regarding scientific equipment and chemicals, the Trade Union Committee for Reconstruction in Vietnam has sent material worth some thousands of pounds to Professor Hieu for use in both the north and south of Vietnam.

On a more institutional level, Professor N. W. Pirie last year visited North Vietnam on behalf of the Royal Society and this year the British Government made a donation of scientific, medical and English literature to three institutes in Hanoi.

Several scientific institutes in the UK have expressed a desire to continue to supply current literature to Vietnam. This could easily be arranged, either through the British Council or the Ministry of Overseas Development, and would not require excessive funds. Our committee put forward these suggestions to both the British Council and the Ministry several months ago and we are waiting for their decision.

Perhaps now that more British scientists are aware of the problems facing their Vietnamese colleagues as a result of Professor Arthur Galston's article, there will be greater developments in the British aid programme to Vietnam.

Yours faithfully,
A. HAY

Medical Aid Committee for Vietnam,
London WC2, UK

Journal guidelines

SIR,—It is good that you saw the purpose of the document on "Authors, Editors and Referees" issued by the Scientific Information Committee of the Royal Society—that is, to initiate well informed discussion on this fundamental element of the communication system of science, and to bring into the open the actual procedures that are used (November 6). Indeed, it is only by showing that these procedures are honest and just, giving a fair weight to the interests of all parties concerned and to the needs of the scientific community at large, that we can justify our resistance to the demand to disclose the names of referees to authors, and similar populist follies.

You are also correct in pointing out that the proposed 'guidelines' are not appropriate for your own journal. We are all happy with the thought that *Nature* is almost unique in the world of science. And I would not apply precisely this code to a review journal such as *Reports on Progress in Physics.* But the question whether editors of conventional primary research journals are well advised to deviate very far from the suggested code is worth open and collective debate and should not be confined to laboratory coffee clubs or to the committee rooms and editorial boards of individual learned societies.

May I suggest that by discussing this document in an editorial, quoting only points with which you disagree, you underestimate the intelligence and good sense of your readers, who might like to decide for themselves whether it had some merit in general. Since the whole 'code' is scarcely longer than this letter, why not publish it in full for wider discussion?

Yours faithfully,
JOHN ZIMAN

University of
Bristol, UK

Here it is:

(i) Every paper submitted to the journal for publication should be refereed.

(ii) Referees are appointed by the Editor, and report to him.

(iii) The name of any Referee may only be disclosed to the Author by permission of or at the request of the Referee, with the Editor's agreement.

(iv) The Referee may not disclose the contents of a paper submitted to him, nor make use of these for his own scientific work before publication, without explicit permission of the Author, to whom the name of the Referee must then be disclosed.

(v) The Referee's report should contain a definite recommendation concerning publication based upon a reasoned judgement of the general form and scientific contribution of the paper as a whole. He may recommend acceptance outright or subject to revision of certain sections along specified lines, or may propose that the paper be substantially rewritten to improve the presentation or to strengthen the scientific argument. The substance of all such critical comments should be communicated to the Author for action, to the eventual satisfaction of the Referee. But failure to accept the Referee's view on all minor points of criticism should not be a bar to publication.

(vi) No paper should be rejected on the adverse report of a single Referee.

(vii) In the case of conflicting opinions, the Editor may appoint further Referees or an Adjudicator who may read all communications between the Author and Referees (whose names need not be disclosed to him).

(viii) A definite procedure should be established for Editorial decision within a stated period.

We disagree with i, vi, and viii (but see our statement of editorial procedures on page 1 of the November 6 issue) and would like to hear what others think.

ED.

news and views

Successional patterns and indices of diversity

from Robert M. May

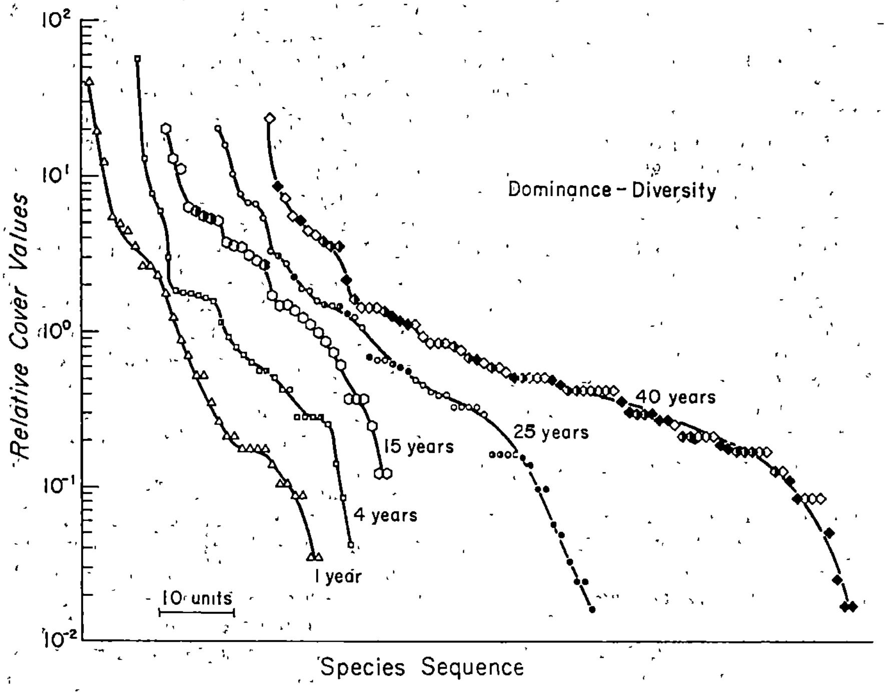
ONE way to quantify Robert Louis Stevenson's belief that "the world is so full of a number of things" is simply to count how many things there are. The result gives the 'species richness', the number of plant or animal species observed to be present in a given community.

But usually one wants to know more than this. If 20 species are present, are they roughly equally abundant, or is one common and the other 19 rare? A full answer to this question involves finding the distribution function which describes the relative abundance of all the species in the community. On the other hand, one may wish to settle for less, and be content with some statistical moment of the distribution, some single number, which characterises the diversity of community. A large (and often muddled) amount of the ecological literature is devoted to the search for such 'diversity indices'. A lucid account of some mathematical aspects of these questions is in the monograph just published by Pielou, *Ecological Diversity* (Wiley; 1975).

Field studies of the relative abundance of species in a variety of plant and animal communities have revealed interesting patterns, many of which can be plausibly explained on theoretical grounds. In the early stages of succession, plant communities will tend to consist of a handful of weedy, pioneer, r-selected species that happened to get there first. The species arriving first will tend to grow rapidly, pre-empting a fraction (*k* say) of the available space or other governing resource before the arrival of the next species, which in turn will tend to pre-empt a similar fraction of the remaining space before the arrival of the third, and so on. The consequent pattern will be, very roughly, a geometric series in relative abundance. As succession proceeds, things become more complicated, and a multitude of ecological dimensions are likely to be relevant to the ultimate composition of the community. That is, in later successional stages the relative abundances of the various species are governed by the interplay of many more-or-less independent factors. It is in the nature of the equations of population dynamics that these several factors should compound multiplicatively, and the statistical Central Limit Theorem applied to such a product of factors implies a lognormal distribution (that is, a bell-shaped gaussian distribution in the logarithms of the species' abundances). Once many species are involved, a lognormal distribution gives a much more even pattern of species relative abundance than does a geometric series distribution: the lognormal is a socialist society compared with the feudal hierarchy of the geometric series.

These notions are in accord with the data obtained from several studies of plant succession at various locations in the temperate zone (see, for example, Whittaker, *Communities and Ecosystems*, ch. 4, second ed, Macmillan, New York; 1975). The patterns show up with particular clarity in the recent study by Bazzaz (*Ecology*, **56**, 485–488; 1975) of old fields in southern Illinois, at successional stages varying from 1 to 40 years. When the logarithm of species relative abundance is plotted against the rank of the species in an abundance hierarchy, early successional stages show the straight line relationship characteristic of a geometric series, while later stages show a progressively more S-shaped curve, with a preponderance of "middle class" species, characteristic of a lognormal distribution. The figure, taken from Bazzaz's paper, makes this plain.

Something akin to a reversal of these successional patterns takes place when mature communities become polluted. This has been shown particularly for streams and lakes subject to "enrichment" by waste heat, sewage, and other materials. The most detailed studies are those of Patrick and coworkers on the changes in patterns of species relative abundance in diatom communities. The equilibrium diatom community essentially always



Patterns of species relative abundance in old fields of five different stages of abandonment in southern Illinois. The patterns are expressed as the percentage that a given species contributes to the total area covered by all species in a community, plotted against the species' rank, ordered from most to least abundant. Symbols are open for herbs, half-open for shrubs, and closed for trees (from Fig. 2 of Bazzaz, *Ecology*, **56**, 487; 1975).

shows the classic lognormal distribution; when polluted, the community typically shows a pattern in which a few species become exceptionally common, with a hierarchical geometric series distribution (possibly caused by an early successional type of r-selection for success in handling the all-important new factor). Closer analysis may, or may not, reveal a lognormal distribution of relatively rare species hanging on down in the tail of the distribution.

These connections between patterns of relative abundance and degree of pollution have sparked off interest in the possibility of using some index of diversity to measure how much an ecosystem has been perturbed by human activity. One such attempt, using a diversity index as a phenomenological measure of gross toxicity in San Francisco Bay, is due to Anderson *et al.* (*Proceedings of 5th International Conference on Water Pollution Research,* Pergamon; 1971). Clearly, an engineering-style index of this kind would simplify tasks of environmental management. But Patrick (private communication) emphasises that, for her diatom communities in polluted streams, any such single number will be dominated by the geometric distribution of the common species, whereas the time scale for recovery of the pristine ecosystem (and, indeed, whether it can ever recover) depends on the lognormal tail of species which are uncommon in the polluted community, and whose presence will not show up in any overall diversity index. This amounts to a cogent argument (similar to that advanced by Williamson, in *The Mathematical Theory of Biological Populations,* Academic Press, New York, 1973) for describing the community by its full distribution of species relative abundance, and not trying to condense information into a single diversity index, which may mislead. The whole subject has practical importance for environmental impact studies, and deserves more attention than it has received to date. □

Earthquake prediction in China

from David Davies

IN March 1966 two severe earthquakes struck Hopeh Province, China; the scene of the disaster (relatively close to Peking) was visited by Chou En-Lai in his capacity as Prime Minister. Fortuitously the early part of 1966 also saw the start of the Great Proletarian Cultural Revolution. Earthquakes in China had caused great loss of life throughout history (nearly a million people died from one in 1556); what more logical project for a rebuilt people-oriented science programme than to attempt to predict earthquakes. And what more logical approach to this problem than to involve the general public as intensively as possible.

Nearly ten years later the fruits of this remarkable enterprise are beginning to become visible to Western eyes. In the November issue of *Eos,* the Transactions of the American Geophysical Union, is presented an extensive report of the American Seismology Delegation of October 1974 to China. Coincidentally, travellers' tales from more recent visitors to China begin to confirm the successful prediction of a very large earthquake on February 4, 1975—a prediction that may well have saved many thousand lives.

China does not fit simply into the scheme of plate tectonics. Scattered continental seismicity did not lend itself to the drawing of unambiguous plate boundaries, and it has taken several years to integrate what is known about surface geology, broad-scale lineations, earthquake distributions and their focal mechanisms into a reasonable tectonic synthesis. The work of Molnar and Tapponnier (*Science,* **189,** 419–426; 1975) suggests, for instance, that much of the seismicity in China can be considered as a result of the taking up of the convergence of the Indian subcontinent on Eurasia at a rate of 5 cm per year, with the concomitant necessity for parts of Eurasia to slide laterally out of the way. One of China's enormous assets is an earthquake record of sorts which goes back 3,000 years. North China in particular, has a record which the delegation called 'remarkably complete' for large events since 466 BC, and homogeneous for even small (magnitude 5) events since 1484 AD. The group noted that comparable dates for southern California would be 1850 and 1930. And yet even with this quality of reporting the patterns still remain complex, and as a result any programme of vigilance has to cover a substantial area of the country.

Ten thousand professionals participate in the Chinese earthquake programme, ten times the number in the United States, and there is a band of many thousand amateurs. Activity is spread widely over the country and the instrumental content of the programme is equally broadly based. Work is being conducted on all types of premonitory symptoms studied in the West—seismic, geodetic, electromagnetic, geochemical, magnetotelluric; and in addition there is considerable interest in acoustic precursors (earthquake sounds) and the unusual behaviour of animals. These last two phenomena have had a very shadowy history in the West—there is practically no scientist concerned about his professional reputation who would have ventured to express enthusiasm for them. But in China, where everyone is encouraged to report unusual events, there is a much greater interest in such possibilities.

Despite the encouragement of central government to pursue science for the people, it took five years from 1966 before the nationwide programme began in earnest. The turmoil at the Academy of Sciences during the cultural revolution must have been one major factor, as there was obviously a lack of steady central administration. Another factor must undoubtedly have been inadequate practical experience amongst geophysicists; the legendary ability of the Chinese has been to solve theoretical problems. Since 1971, however, the programme has developed enough confidence to issue at least eleven predictions which have led to extensive evacuations of threatened regions. Precise details both of the success rate and of the total data sets used have not been released, but there is no doubt at all that there have been major successes.

Ironically three months after the delegation had left China the most spectacular prediction came, for which, as yet, we have only the reports of more recent visitors. It seems that Liaoning Province had for several years been viewed as a possible target for a major earthquake as a result of migration of seismicity, and in the months before the quake, tilting occurred and there were observations of changes in well levels, telluric currents and animal behaviour. Then immediately before the magnitude 7.3 event, foreshocks gave sufficient warning for mass evacuations. There was enormous damage—a town of 100,000 is being completely rebuilt—but few fatalities.

Satisfaction at this major success must be tempered with some concern over the way in which the work will now advance. One of the disturbing themes running through the American delegation's report is an apparent weakness in more basic fields of research. The pendulum seems to have swung so far from theory that it is questionable whether there is adequate work going into the fundamental understanding of the processes that are being observed. Visitors speak of a far too ready acceptance of the work of any and everybody without the profound criticism that advances science most rapidly.

The successes of the Chinese programme come at a particularly crucial stage in the development of the United States project under the auspices of the Geological Survey. Recently an increasing amount of attention has been paid to the public policy aspects of prediction with, at least in some people's opinion, a concomitant build up in the public expectation that scientists are going to be able to deliver the goods in the very near future. Yet many seismologists privately entertain serious doubts that the premonitory symptoms are anything like unambiguous enough to warrant going public for some years to come. Three weeks ago Dr V. E. McKelvey, Director of the Geological Survey, was saying at a conference on earthquake warning and response, 'we are now entering an age when scientific instruments are detecting geophysical signals that can be interpreted to forecast earthquake occurrence'. Many would have preferred him to have put this age five or ten years in the future while some very difficult work is being done. Unfortunately, although the Chinese results are very exciting indeed, they may give the false impression that the United States is in for a similar pay off. This is rather unlikely. Foreshocks, the means by which the Chinese pinned down the time of the quake so accurately, are a relatively rare occurrence before major events, and other techniques used such as telluric currents, migration of seismicity and radon levels in ground water have yet to show their usefulness in the United States.

One thing is indisputable, however. The Chinese work will have acted as a shot in the arm to seismologists in all other countries engaged in prediction research. And by all accounts there is more excitement still to come. □

Messengers in minicells

from M. R. Blundell

THE first experiments demonstrating the existence of messenger RNA in bacteria were published in 1961. One of the criteria for identification of mRNA was its short lifetime, which permits a rapid halt in synthesis of a specific protein, but our knowledge of the processes responsible for inactivation and degradation of mRNA remains very incomplete fourteen years later.

We know that degradation proceeds from the 5′ to the 3′ end of mRNA (Morikawa and Imamoto, *Nature*, **223**, 37, 1969) but it is clear that the 5′-exonuclease activity called ribonuclease V (Kuwano, *et al.*, *J. molec. Biol.*, **51**, 75; 1970) cannot be responsible alone for mRNA degradation (Imamoto and Schlessinger, *Molec, gen. Genet.*, **135**, 29; 1974).

What was regarded as merely a scavenging process has taken on new significance with the discovery that individual messages in the same *Escherichia coli* cell can have different decay rates with half-lives ranging from 40 s upwards at 37 °C (Marrs and Yanofsky, *Nature new Biol.*, **234**, 168; 1971; Blundell *et al.*, *Nature new Biol.*, **238**, 46; 1972; Craig, *et al.*, *J. molec Biol.*, **71**, 701; 1972, and others). Even messages in the same polycistronic mRNA can have quite different decay rates.

Hirashima, Childs and Inouye (*J. molec. Biol.*, **79**, 373; 1973) discovered a message with a particularly long functional half-life, 11.5 min at 37 °C, which codes for a low molecular weight lipoprotein of the outer membrane of *E. coli.* Both *et al.* (*J. molec. Biol.*, **67**, 199; 1972) presented measurements of the secretion of an extracellular protease by *Bacillus amyloliquefaciens* which could be interpreted in terms of a mRNA stable for up to 80 min at 30 °C, but subsequently, they concluded (Glenn *et al*, *J. molec. Biol.*, **73**, 221; 1973) that this message was not especially stable, but was instead made in very large quantities. In hybridisation experiments, however, Brown and Coleman (*J. molec. Biol.*, **96**, 345; 1975) could not find such quantities of mRNA, or any especially stable mRNA in cultures of *B. amyloliquefaciens.*

Now Levy (*Proc. natn. Acad. Sci. U.S A* , **72**, 2900; 1975) reports the presence of a small quantity of very long lived mRNA in *E. coli* minicells, structures about one tenth the size of whole cells which are made in abnormal cell divisions of an *E. coli* mutant and lack the *E. coli* chromosome. Plasmid DNA that segregates into minicells is transcribed and translated, but plasmid-less minicells do not make DNA or RNA. Levy observes, however, that ^{35}S-methionine is incorporated by freshly isolated minicells into hot trichloroacetic acid precipitable material at 0.2 to 0.5% of the rate in whole cells. The incorporation is sensitive to chloramphenicol, so it is presumably ribosomal polypeptide synthesis. Protein synthesis decays during incubation at 37 °C with a half-life of about 80 min, and is unaffected by rifamycin. The major products of protein synthesis in plasmid-free minicells are four polypeptides of the outer cell membrane, one of these being the lipoprotein coded by the long lived mRNA studied by Hirashima *et al*

We can, therefore ask, as does Levy, why the messages for membrane proteins should be so stable in minicells. Minicells containing an R factor continue to make short lived mRNA which codes for other proteins, so mRNA can be degraded in minicells, but no comparison of the decay rates of R factor messages in whole cells and minicells is presented. One possibility, since minicells form from the poles of cells, is that the cell extremities, and therefore minicells, are relatively deficient in specific nuclease(s).

We may also ask why membrane proteins should have abnormally stable message. To investigate this question Hirashima, Wang and Inouye are now developing a cell-free translation system which synthesises the lipoprotein when purified mRNA is added. □

Unexpected proviruses in chronic infection

from Robin Weiss

RECENT reports by Zhdanov (*Nature*, **256**, 471; 1975) and by Simpson and Iinuma (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 3230; 1975) promise to open up a new area of genetic exchange between RNA viruses and their hosts. These authors claim that some animal RNA viruses that conventionally replicate by means of RNA intermediates can under conditions of chronic infection form DNA "proviruses".

Simpson and Iinuma have studied a temperature sensitive mutant of respiratory syncytial (RS) virus in bovine embryo kidney (BEK) cells infected and maintained at the non-permissive temperature of 39 °C. These BEK/RS cells did not release RS virus or synthesise RS viral antigens detectable by immunofluorescence. However, even late passage cultures yielded infectious virus when co-cultivated with uninfected cells or when treated with agents such as halogenated pyrimidines. These findings led the authors to look for DNA proviruses of RS virus using transfection techniques as described by Hill and Hillova (*Nature new Biol.*, **231**, 261; 1971) for the provirus of Rous sarcoma virus. When HEp-2 cells were treated with DNA extracted from the BEK/RS cell line infectious RS virus was recovered. A clonal analysis of the progeny RS virus indicated that some clones retained the special properties of the virus that initiated the chronic infection—temperature-sensitive replication and heat-resistant virions—whereas other clones had lost one or both of these properties and more closely resembled wild-type RS virus. Simpson and Iinuma further showed that a chronically-infected cell line of HEp-2 cells, survivors of an infection with wild-type RS virus, similarly contained infectious DNA for RS virus.

Zhdanov's analysis is less detailed but wider ranging, and illustrates the same phenomenon of DNA provirus formation in cultures chronically infected with RNA viruses. Persistent infections were established with measles virus in chick embryo cells, Sindbis virus in murine L cells and tick-borne encephalitis virus in HEp-2 cells. Using tritiated viral RNA, Zhdanov showed by hybridisation C_0t analysis that the chronically infected cells in each of the three systems contained sequences homologous to the viral RNA, whereas the uninfected control cells did not. With the chronically infected HEp-2 cells he was also able to recover infectious tick-borne encephalitis virus by DNA transfection.

The formation of proviruses demands transcription of RNA to DNA and both groups found 'reverse transcriptase' activity associated with the chronically infected cells. Zhdanov assumes that the persistently infecting virus interacts with latent oncornaviruses present in the cultures. It will be interesting to determine whether the presence of oncornavirus reverse transcriptase is necessary for provirus formation, and whether the provirus is a prerequisite to or a consequence of the development of such persistent infections. What is remarkable is that not only are some DNA sequences transcribed, but whole, transfectable proviruses are formed, suggesting a process of orderly, complete reverse transcription. Simpson and Iinuma also comment in their discussion that plaque-purified RS virus recovered from DNA transfection carries reverse transcriptase activity and may represent recombinant virus species. One wonders, therefore, whether other lytic viruses which replicate by way of a DNA provirus, such as visna virus and avian reticuloendotheliosis virus, might have evolved in a similar way and might possess alternative modes of replication.

These studies raise interesting questions not only for molecular biologists but also for students of persistent infections and 'slow' virus diseases. Zhdanov presents evidence for measles virus DNA in tissues of patients suffering from systemic lupus erythematosus. No doubt the hunt is on for DNA copies in other 'viral' autoimmune diseases. □

Martian canals after Mariner 9

from David W. Hughes

EVER since 1877 when Schiaparelli drew attention to the existence of canals on Mars they have been the subject of heated controversy and, at times, distinct embarrassment to planetary astronomers. Mariner 9, the spacecraft that went into orbit around Mars in 1971, achieved a complete mapping of the surface down to a resolution of 1 km, so now the question, "where are the canals?" should be answerable. Sagan and Fox of the Laboratory for Planetary Studies, Cornell apply themselves to this question in a recent issue of *Icarus* (**25**, 602; 1975).

Many suggestions have been put forward to explain the observations, observations which cannot be discounted out of hand, because, even though the features are of intrinsically low contrast and small angular size usually observed under poor seeing conditions, many maps of tolerable mutual consistency have been produced by different astronomers.

Canals have been thought to be waterways or the vegetation along the banks of waterways constructed by an advanced civilisation. Natural valleys, surface cracks or ridges produced by a range of geological processes were another suggestion. Accidental alignments of surface features (for example chains of large craters) was a third. They may also be rectilinear sand dunes or dark rays emanating from craters, or simply albedo features, regions of differing reflectivity that are periodically covered and uncovered by windblown dust.

Sagan and Fox compare the Mariner 9 results with the canal cartography of Slipher (*Mars,* Sky Publishing Co., Cambridge, Mass; 1962). A striking correlation was found between the canal Agathodaemon and the great rift valley Valles Marineris but as the latter is a low albedo feature 5,000 km long and 100 km wide it is only to be expected that this is easily resolvable by ground-based observers. Thoth-Nepenthes is also easily discernible on both maps as is the ridge Ceraunius.

Returning to the hypotheses concerning canal origin, Sagan and Fox conclude that the resolution and surface cover of Mariner 9 would have detected a civilisation like Earth's if it had been present on Mars. Correspondences do occur between classical canals and valleys or ridges, Agathodaemon-Valles Marineris has been mentioned and Styx-Phlegra Montes and Ceraunius are other examples. Psychophysiological alignments of disconnected fine detail only seem probable in the case of the canal Oxia which seems to be the chain of craters Trouvelot, Rutherford, Becquerel, Curie and Sklodowska and an unnamed canal which goes through craters Galle, Wirtz, Helmholtz and Lohse. Very few crater rays were seen by Mariner 9 and sand dune fields do not seem to be sufficiently extensive to be observed from Earth. A few canals notably Cerberus and Thoth-Nepenthes do correspond to dark features on the Martian surface, these features being unconnected to topography. They might, however, be regions where fine bright dust does not settle or regions where the ground is dark due to surface roughness or some geochemical reason.

While a few canals do correspond to topographic and albedo features on Mars, Sagan and Fox conclude that the large majority of canals do not. Conversely there are many real surface features, for example large shield volcanoes like Olympus Mons, Arsia Mons and Pavonis Mons, where no canals were marked on the maps.

Sagan and Fox discount the canal observations and relegate them to monuments to the imprecision of the human eye labouring under difficult seeing conditions. But does the story really end here. Probably so, but the consistency of the diverse maps leaves a lingering unease. Maybe the facts that Mariner 9 could not distinguish between features which had less than 5% albedo contrast, that Mars was only observed for half a Martian year, or the violent dust storms that preceded Mariner 9's visit caused the real truth to be hidden. □

Parity determination with polarised deuterons

from P. E. Hodgson

A NEW technique for determining the parity of nuclear states has been developed recently by Kuehner *et al.* (*Phys. Rev. Lett*, **35**, 423; 1975) at McMaster University, Hamilton, Ontario. The method depends on the properties of the coupling between the orbital and spin angular momentum vectors in the initial and final states. In certain conditions the yield of particles from a reaction at 0° (or 180°) on an even-even target is zero for states of natural parity $(-)^J$, so a single measurement determines the parity if the spin J of the state is known.

The conditions for this to hold are that the magnetic quantum numbers m are zero in the initial and final states. Since these are the z-components of angular momenta (taking the beam direction as the axis of quantisation) this may be ensured by using a polarised incident beam with $m=0$ and detecting the products of the reaction along the beam axis, that is at 0° or 180°. Nucleons have $m=\pm\frac{1}{2}$, and so cannot be used, but deuterons having spin one can be in the $m=0$ or ± 1 states, and it is possible to polarise the beams.

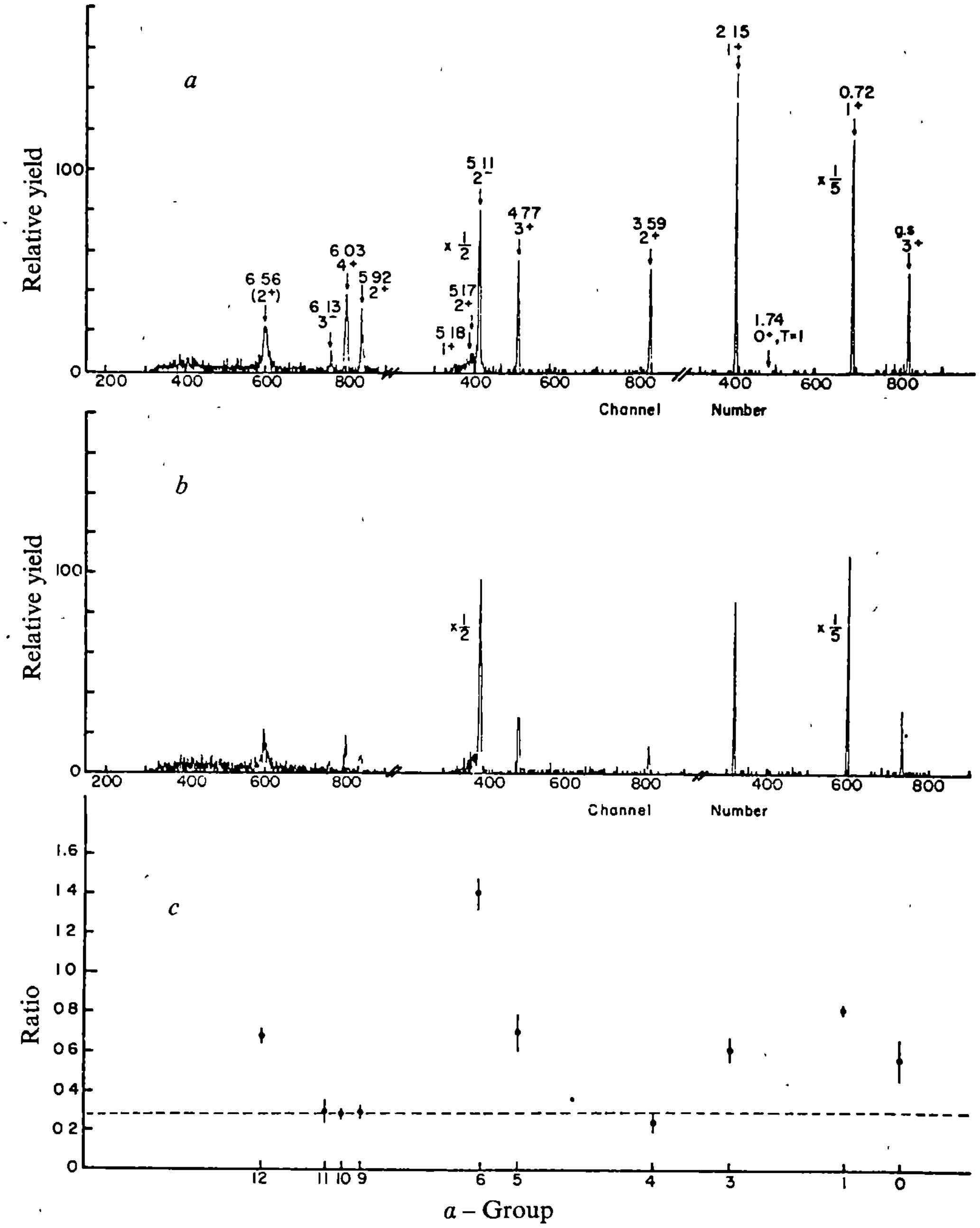
Although this method is simple in principle, it is not simple to carry out in practice. It is difficult to produce polarised beams, and existing techniques cannot yet give 100% polarisation. It is therefore necessary to compare the results of measurements with unpolarised and partially polarised beams. It is also difficult to make measurements very near to 0°, but a subsidiary study showed that in practice the intensities of the forbidden reactions are very low below 2°, and so measurements made at this angle are sufficiently good.

Kuehner *et al.* applied this technique to the reaction $^{12}C(d,\alpha)\ ^{10}B$, and some of their results are shown in the figure. The upper spectrum was obtained with an unpolarised beam and the lower with a partially polarised beam. It is immediately evident that the relative intensities are different, and the actual ratios are also displayed. The intensities of the states with parity $(-)^J$ are most strongly reduced, though not to zero, because of the residual $m=\pm1$ components in the partially polarised beam. A subsidiary calculation shows that these states should be reduced by the ratio indicated by the dashed line in the figure.

These ratios therefore give immediately the parity of the final state of ^{10}B: those with ratios on the dashed line have parity $(-)^J$ and those with ratios above the line have parity $(-)^{J+1}$. Since the spins are usually known, this gives the required parities. There may be some cases where the parity is known and the spin from other measurements must have either of two consecutive values; in this case the method determines the spin.

An example of this latter situation is provided by the state of ^{10}B at 6.56 MeV. Previous measurements had indicated 2^+, though 3^+ was also a possibility. The measurements of Kuehner *et al.* show that the spin must be odd, and thus the assignment 3^+ is confirmed.

This new technique should prove a powerful way of determining the parities or spins of nuclear states. If both the spin and the parity are known, it can be also used to determine the tensor polarisation of a beam of spin-one particles; this could be particularly useful for projectiles like ^6Li for which the usual double-scattering method is difficult. □



Spectra of alpha-particles from the reaction $^{12}C(d,\alpha)\ ^{10}B$ at 2° to the incident beam for an unpolarised (*a*) and for a partially polarised beam. (*b*) Also shown (*c*) is the ratio of the intensities for the two cases; this has the value shown by the dashed line for states with parity $(-)^J$ and a higher value with states with parity $(-)^{J+1}$.

Seed dispersal

from Peter D. Moore

SEED production, seed germination and seedling establishment are, for most plant species, critical stages in the maintenance of population levels. The quantity of seed produced and the energy reserve of each individual seed vary from one species to another;

Harper, *et al.* (*A. Rev. ecol. Syst.*, **1**, 327; 1970) pointed out that r-selected species often produce large quantities of small seeds whereas K-selected ones often have a small number of large seeds. Survival of seeds depends upon the avoidance of predation, and numerous mechanisms have evolved by which such avoidance may be effected, for example by toxicity or by rapid and widespread dispersal (Janzen, *Evolution*, **23**, 1; 1969). The scattering of seeds besides reducing the risks of predation, also assists the avoidance of intra-specific competition among seedlings and permits the spread of the species to new localities, thus aiding the exchange of genetic material.

A recent study of seed production and dispersal in the genus *Viola* by Beattie and Lyons (*Am. J. Bot.*, **62**, 714; 1975), illustrates the complex results produced by these interacting selective pressures. Initial studies of seed dispersal in the genus led to the classification of *Viola* species into two major groups, one myrmecochorous (dispersed by ants) and the other diplochorous (explosively ejected from the capsule and subsequently further transported by ants). Diplochorous types have many morphological features associated with projectile dispersal, such as an erect, tall peduncle to the capsule and a relatively small caruncle on the seed when compared with the myrmecochorous types. The explosive ejection of these seeds often results in their being thrown 1·5 m from the parent plant.

The possession of a caruncle by the diplochorus types suggests that there is a subsequent stage in dispersal when the caruncle may serve as an attractant and a food reserve for the transporting agent. If this were not so, then selection would presumably have resulted in the loss of the caruncle in these species in the interest of aerodynamics. To examine this, Beattie and Lyons placed different types of *Viola* seed in areas of woodland in West Virginia and also in prairie in Illinois where ant nests were abundant. The diplochorous seeds were collected by ants when fresh whether they were placed in groups or scattered as individuals, but the rate of collection was considerably lower if seeds from the previous season were used. Evidently their attractiveness to ants is adequate while they are fresh, but deteriorates with age.

In the case of the myrmecochorous types, only old seed was available. These were placed in groups to simulate the natural situation when such seeds would remain in their capsules. It was found that the rate of disappearance was as rapid for these old seeds as in the case of the fresh diplochorous seeds. The myrmecochorous types evidently have mechanisms of attraction which are more persistent than those of the diplochorous types.

The myrmecochorous types have evolved a dispersal strategy in which they combine tough seed coats, easily accessible energy-rich seeds located in groups, with a frequent recurrent production of new seeds. The danger of such a strategy lies in the close grouping of the seeds, which exposes them to predation, and this is overcome by the development of mutualistic relationships with specific ant species. These ants collect the seeds regularly from predictable localities and carry them to their nests where they feed upon the caruncle, leaving the embryo unharmed and able to germinate. Predation avoidance is thus dependent upon collection and dispersal by ants. The heavy seeds and large, energy-rich caruncles of these species assist in the strategy, which can be considered as an essentially K-selected one.

The diplochorus mechanism provides an alternative solution to the dangers of seed predation. Local concentrations of seeds are avoided by the explosive dispersal mechanism, but the retention of the caruncle ensures that the seeds will be found and further dispersed by ants. In the experiments, almost all fresh diplochorous seeds had been found and removed within three days. Beattie and Lyons suggest that full dependence on ant dispersal is only possible in situations where an ant species with the appropriate behaviour pattern is abundant. In sites with depauperate ant faunas, the diplochorous strategy is more reliable. This may account for the geographical restriction of the myrmecochorous *Viola* species to those regions of Eurasia where the mound-building, scent-trail laying ant species *Formica rufa* is found. □

Fishing for complement

from Robin Hesketh

THE study of the movement of protein complexes in the plane of the fluid membrane has kept a good many immunologists off the streets since the demonstration in 1971 by Taylor, Duffus, Raff and de Petris of the redistribution of lymphocyte surface immunoglobulin molecules after their interaction with antibody It now seems that a venerable method for measuring the binding (fixing) of complement (C) by antigen–antibody complexes may assist in defining the necessary environment for membrane receptors to participate in some immunological reactions.

It is now well known that the fluidity of membranes can be changed by varying the amount of cholesterol present, and Humphries and McConnell (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2483, 1975) have used this fact to correlate the lateral mobility of a membrane antigen with its C-fixing ability.

As a model membrane they used a lecithin bilayer containing cholesterol into which the monovalent lipid antigen cardiolipin had been introduced. Complement binding by this system in the presence of human syphilitic serum, which contains antibodies against cardiolipin, provides one of the simplest models for immune cytotoxic attack.

This system of lipid suspensions, later christened liposomes, was first developed by Osler and Knipp (*J. Immun.*, **78**, 19; 1957) in a search for a precise method for estimating the cardiolipin-specific Wasserman antibody in human syphilitic serum. They showed that the low antigenic activity of suspensions of monovalent cardiolipin was greatly enhanced by the addition of lecithin, and that maximum precipitation of antibody occurred when cardiolipin was present with lecithin and cholesterol.

In measuring C fixation, Osler and Knipp showed that rabbit or human syphilitic sera yielded identical reaction curves with three different mammalian cardiolipins, and that dimyristoyl lecithin could substitute for beef heart lecithin in the liposome without reducing its ability to bind IgM and C. They also showed that the lecithins alone had very little anti-complementary activity.

Using dimyristoyl or dipalmitoyl lecithin in the cardiolipin-sensitised lipsome system of Osler and Knipp, Humphries and McConnell found that the degree of C fixation increases with the rigidity of the membrane (that is, with cholesterol concentration). A minimum cholesterol content of 35 mol %, however, is required to initiate significant C consumption. It is known from X-ray diffraction and differential scanning calorimetry that phospholipid/cholesterol interaction is maximal at 33 mol %—greater cholesterol concentrations creating cholesterol–cholesterol contacts within the membrane. Humphries and McConnell have used electron spin resonance methods to construct a phase diagram for the lipid systems used which indicates that at greater than 35 mol % cholesterol the cardiolipin becomes strongly immobilised and probably laterally segregated—either in an environment consisting entirely of cholesterol or incorporated in a solid phase of the membrane. The authors suggest that cardiolipin 'receptors' in this state may be able to bind several of the sites on an IgM molecule. thereby distorting the structure of the antibody in the manner required to accommodate the first component of the complement sequence. This conforms with the hypothesis of C fixation by IgM propounded by Ishizaka and Ishizaka (*J. Immun.*, **102**, 1337; 1969) and supported by electron micrographs of 19S haemolytic antibodies forming loops to bind to nearby sites on a single erythrocyte membrane (Humphrey and Dourmashkin, *Ciba Foundation Symposium on Complement*, 1965).

Returning to the wider implications of their work, Humphries and McConnell, although aware of course that submembranous structures as well as lipid composition are involved in protein mobility in membranes, observe that the conditions of hapten mobility which they find necessary for C-mediated cytotoxic attack may also apply to direct lymphocyte stimulation by these liposomes. As evidence for this they point out that haptens stimulate an immunological response only when they are bound to a protein carrier. Such intriguing parallels will no doubt leave cellular immunologists asking how long it will be before the complementary experiments of manipulating the composition and hence the mobility or function of receptors is accomplished in a living cell. ☐

Circadian rhythms

by Miranda Robertson

The Dahlem Workshop on the Molecular Basis of Circadian Rhythms was held in West Berlin on November 3–7.

With a field in the state that prevails in research on circadian rhythms, the particular format of the Dahlem Konferenzen should have proved an interesting experiment. The Workshops, of which this was the third, are explicitly designed to define the most important problems in a given field and to suggest fruitful directions for further research. Most of the conference time is spent in discussion: instead of being formally presented, background papers are written by and distributed to participants before the beginning of the meeting, and by the end, the members of each of six small groups have been forced into some kind of agreement in the production of a group report on their conclusions.

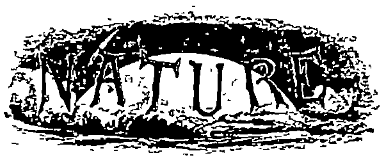
Agreement is a rare commodity in this field, whose present state has been compared (optimistically?) with that of Mendelian genetics before the discovery of the genetic code in the sense that the "hard" data are phenomenological. It is now very well established that numerous physiological processes, from photosynthesis in plants to motor activity in mammals, have an autonomous more-or-less 24-hour cycle which can be synchronised with the diurnal light-dark cycle by entrainment. The phenomenology has been enormously extended in experiments on how the various rhythms respond to changes in the phase or the period of the entraining cycle (the Zeitgeber). The task of the workshop participants was to decide on a strategy for discovering what cellular mechanism might give rise to oscillations with the observed characteristics. A group of mathematical modellers tried to decide what those characteristics say about how the clock works in principle and concluded that it is still impossible to distinguish between mechanisms as simple as the "dripping faucet" and as sophisticated as a limit cycle. Indeed, in general the field suffers from a surfeit of models and a dearth of critical experiments Three categories of nonmathematical model were represented: enzyme models, genetic models and membrane models. The evidence in favour of any of them however tended to be somewhat general: substances which, say, disrupt protein synthesis or alter membrane characteristics sometimes disrupt the circadian rhythm one way or another.

One serious problem facing genetic and membrane modellers is that neither genetic regulation nor membrane function is properly understood in eukaryotic cells.

Another more general problem is that most researchers now agree that it is highly unlikely that there is any single clock mechanism. (Thus the analogy with Mendelian genetics is weak and Colin Pittendrigh's (Stanford) hopes of a "clock phage" doomed to disappointment.) Yet the models are built on the basis of data gleaned from the entire flora and fauna of biological horology, and the confusion is most apparent when anyone attempts to rule out any one possibility. For example, the importance of Ashkenazy's recent discovery is supposed to be that it eliminates genetic mechanisms, since circadian oscillations in enzyme activity seem to persist in red blood cells and even their isolated membranes. But strictly, it rules out genetic mechanisms only for red blood cells.

This is a curiously tantalising field, in which promising leads seem to turn into blind alleys. The single-celled gonyaulax, for example, manifests circadian rhythmicity in three different physiological mechanisms, all of whose rhythms can be affected by a single mutation. Clock mutants are known in several other species, including two of *Drosophila*. Since both the genetics and the circadian phenomenology of *Drosophila* are so well known, one would expect such mutants to offer a golden opportunity for research.

It does seem, however, that the field would be best served if people stopped looking for "the" circadian clock and focused on that of one particular organism. It may not turn out to be a 'phage' but one well known mechanism could nonetheless prove in the end more illuminating than any number of plausible models. ☐



M Charbonnier advocates . . . the theory that people may subsist without food because the nitrogen from the air can be admitted into the circulation system, when the body has been emaciated by long abstinence. Feeding on air is an economical way of keeping soul and body together

from *Nature*, **13**, 95; Dec. 2, 1875.

Sperm egg interaction

from a Correspondent

The First Symposium of the International Society for Invertebrate Reproduction (ISIR) was held at Calicut on September 10–12, under the auspices of the University of Calicut and the Kerala Academy of Biology. Abstracts have been published in *Invertebrate Reproduction*, (edit by Adiyodi, K. G., and Adiyodi, R. G., Publication Division, ISRPI Secretariat, Calicut University, Kerala, India).

Nineteen out of twenty animal species on our planet are invertebrates but it is probably no exaggeration that nineteen out of twenty papers published on reproduction deal with vertebrates. As knowledge of reproductive processes and mechanisms is essential for animal management and invertebrates as a group are at least as vital to human welfare as vertebrates, the Calicut Symposium therefore, marks a welcome departure.

Contributions to the symposium covered many aspects of sexuality and reproduction in invertebrates, such as sexual physiology and population dynamics, spermatogenesis, sperm egg interactions, ovarian cycles, chemistry of egg envelopes, nature of accessory sex glands and their secretions, reproductive behaviour and control mechanisms. The ground covered was so diverse that emphasis will be laid here only on contributions in sperm egg interaction.

Dr Richard L. Miller (Temple Univer-

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sity, Philadelphia) suggested that low-molecular weight species-specific attractants produced by eggs or egg-related structures to draw the sperm toward the egg may occur in most animal groups. Sperm chemotaxis is known to exist definitively only in some sessile Hydrozoa (Cnidaria) and Urochordata. Circumstantial evidence for sperm chemotaxis exists in Calcarea (Porifera), Cephalopoda (Mollusca), Lophophorata (Bryozoa), Chaetognatha, Echinoidea (Echinodermata) and Vertebrata. Chemotaxis of sperm occurs in eggs with simple jelly coat (*Hydractinia*) or with multi-component investments (tunicate eggs). Miller called for more studies on the production characteristics and effectiveness of these chemical messengers and also on sperm response and the effects of these substances on fertilisation rate. R. W. Atherton (University of Wyoming, Laramie) in his contributed paper on neurochemical regulation of sperm motility hypothesised that the effects of neurochemicals on sperm motility in both invertebrates and vertebrates are apparently active through a common pathway linking acetylcholine and acetylcholinesterase to adenylate cyclase activity. Acetylcholine stimulates adenylate cyclase activity and cyclic AMP increases sperm motility.

M. G. Kleve (University of Houston, Texas) described the elaborate centriolar specialisation in spermatids of *Hydractinia* during spermatogenesis and suggested that pericentriolar processes may play an important role in the chemotactic behaviour of the hydrozoan sperm. Wallis H. Clark, Jr , (University of Houston, Texas) said that the cortical rods in the eggs of penaeid shrimps are composed of neutral mucopolysaccharides, and that their release and dissipation are influenced by Mg^{2+} ions, a protease and possibly a phosphatase.

Maturation of starfish oocytes is induced by 1-methyladenine (1-MA) produced by the follicle cells under the influence of a neurohormonal peptide. S. Hirai (Tohoku University, Aomori City, Japan) presented evidence to suggest that cytoplasmic maturation on the oocyte surface responsible for the formation of fertilisation membrane is induced by 1-MA. Mixing of germinal vesicle material with ooplasm, though not a prerequisite for fertilisability of egg cytoplasm is essential for cleavage and in the swelling of sperm nuclei

Composition of the jelly coat of certain marine eggs has evoked great interest in view of the role of the jelly substance in fertilisation. K. Hotta (Kitasato University, Kanagawa, Japan) discussed the isolation and characterisation of the main sialoglycoprotein (about 70% sialic acid) from egg jelly coats of three sea urchins, *Pseudocentrotus, Anthocidaris* and *Hemicentrotus.* Jelly substance in sea urchin eggs has the ability to agglutinate only homologous sperms, suggesting that species specificity of the jelly substance has a chemical basis. Sialoglycoproteins purified from egg jelly of the three sea urchins by Hotta were distinct and species specific.

Investigations on paedogenesis being few, D. F. Went's (Swiss Federal Institute of Technology, Zurich) report on his success with *in vitro* culture of ovaries of the paedogenetic gall midge, *Heteropeza pygmaea* through successive generations was received with great interest. Sex hormones being practically unknown in insects, it should be possible now to study whether juvenile hormone and ecdysone have any role in determining the sexuality in paedogenetically produced eggs.

To the nearly fifty invertebrate sexual and reproductive biologists it was a week well spent in the ancient city of Calicut, which derives its name from calico it used to export centuries ago. Invertebrate reproductive biology seems to have come to stay as a coherent discipline: the second symposium of ISIR is to be held in the United States in three years time.

Comparative biology of skin

from R. I. C. Spearman

A symposium on the comparative biology of skin was held by the Zoological Society of London on October 30 and 31. The proceedings will be published for the society by Academic Press.

The skin surface is the main site of contact between animals and a varied and often hostile environment. In addition to having to adapt to external conditions the integument is sometimes involved in skeletal support of the body as in arthropods with their hard cuticles and nematode worms in which the collagenous cuticle has to withstand quite high internal pressure as in a balloon. How species have coped with these varied conditions is reflected in their skin structure and functions which was the underlying theme of a highly successful symposium. This was the first conference to include both invertebrate and vertebrate skins and it provided a useful forum for the exchange of information between zoologists, entomologists, parasitologists, physiologists and dermatologists. The long periods provided for general discussion at the end of each day were particularly valuable.

K. Simkiss (Reading University) and K. M. Wilbur (Duke University) discussed the functions of mucus secreted by the molluscan skin. Mucus is important in locomotion in land snails and the mucus secreted has been found to have peculiar physical properties; at one stage being watery and at the next stage viscous. The rapid changes in consistency are suggestive of a thixotrophic gel, the viscosity of which is effected by agitation of the molecules. It was suggested that the peculiar visco-elastic properties of mucus are important both in locomotion and in its use as a transporting vehicle in association with ciliary activity. Clearly this concept has wider implications extending possibly to mammalian ciliated mucous epithelia. For special purposes such as transport of eggs, chemically different mucuses are thought to be secreted and K. S. Richards (Keele University showed several histochemically different types of mucous cells in the epidermis of oligochaete worms.

Mucus secretion is important in the skins of fish and amphibia but with the appearance of keratinisation this function of skin has been lost in mammals. H. Fox (London University) outlined the epidermal changes in a metamorphosing frog from the ciliated epidermis of the young tadpole to the keratinised epidermis of the adult. As new types of cells are differentiated others are lost by autolysis or by cornification and sloughing of the dead cells.

The sloughing mechanism of the horny layer in toads and its hormonal control was investigated by P. E. Budtz (Copenhagen University). Mineralocorticoid hormone from the adrenal cortex determines sloughing in anura but the ability of the target epidermal cells to respond is dependent on the stage that they have reached in their intrinsic cycle of growth. Thus homografts of toad skin from donors with their moulting cycle out of phase with that of the recipient failed to respond to circulating hormone and moulting of the homograft remained out of phase in subsequent cycles.

E. Johnson (Reading University) spoke of seasonal differences in the hair morphology in deer. The winter coat contains larger medullary air spaces than the summer pelage and is important for thermal insulation in the absence of under fur in cervidae. Possible hormonal control was discussed by J. Ebling (Sheffield University).

At the close of the conference the possibility of forming a society to hold further meetings on comparative skin biology was discussed. □

review article

Colour centres past, present and future

P. D. Townsend*

In this brief review of the role of imperfections in materials, particular emphasis is given to the development of devices involving insulators and semiconductors. The central role of imperfections in modern technology is demonstrated by the fact that the "academic" study of colour centres is essential even in the field of fusion reactors.

SOLID state physics means many things to many people and in various guises forms the backbone of modern technology. Yet the perfect solids on which undergraduate studies of the field are based do not exist and we must manage with imperfect materials. The forms of the imperfections are much more numerous than is suggested by the common division into "point" or "extended" defects. These terms cover a wide range of lattice disorders; point defects refer to errors in the atomic arrangement or impurity atoms which influence a few lattice sites, extended defects to grosser faults in the lattice which include planes of extra atoms, dislocations, precipitates or phase changes. Some typical crystalline defects are listed in Table 1.

The concept of imperfections in a lattice is quite simple but progress in understanding them to the stage at which we can make use of the modified lattice properties to which they lead has been very slow. There are at least two reasons for this. The first is that in practice the problem is often complicated by interactions between different defects. The second is not a practical problem, but arises from the feeling of many academic physicists that imperfection studies are not intellectually challenging. Inevitably this attitude produces undergraduate courses which minimise the importance of defects, and perpetuate the myth of perfect solids. (Even semiconductor physics is often presented as though the impurity atoms were the only defects.) Semiconductors represent just one area of technology which is dominated by imperfections. The list of examples in Table 2 shows that we can separate areas where the major imperfections are the result of impurity atoms; impurities combined with radiation damage; or effects solely of the changes caused by radiation damage to the "pure" lattice. Such a list of applications does not necessarily point the way to future developments; progress so far has often been a mixture of intuition and empiricism. For example, we still do not fully understand the mechanism of the photographic process although it has been in use for over a century[1].

To emphasise the property changes which can result from imperfections, and the devices which exploit them, I will select examples from colour centre research[2-6]. This field is commonly regarded as a fringe area of solid state physics concerned with the effects of X rays on alkali halides. In fact the issues range more widely. For example, among the suggested methods for obtaining energy from nuclear fusion is a system which uses optics and pulsed lasers to trigger the thermonuclear reaction. This entire system will be in a radiation environment and, as I shall discuss, will rapidly fail unless the problem of colour centre formation is overcome.

Nature of colour centres

Colour centres represent a special case of defects found in transparent materials. Perfect crystalline solids are commonly described in terms of bands of allowed energy states which extend throughout the crystal Insulators, semiconductors and metals differ according to this picture only, in the relative concentrations of electron and unfilled states which exist in the highest occupied band of levels. Imperfections, either intrinsic (that is, from disorder of the lattice atoms or the occupancy of lattice sites) or extrinsic, from the presence of impurity atoms, introduce localised energy levels and if these lie within the forbidden energy gap they provide a means of controlling the electrical properties of the solid. For example, the highest occupied electron level of an impurity atom may occur between the valence and conduction bands. Several such examples of interband energy levels are shown in Fig 1. In the first example, *A*, the electron can be promoted to a higher localised state of the defect but in example *B*, the localised electron can enter the conduction band. This is of great importance for semiconducting materials such as silicon where the conduction band is essentially empty at room temperature but can be controlled by electrons which are optically or thermally stimulated from defects of type *B*. Figure 1 also indicates electron transfer to a deeper level which could be accompanied by luminescence.

Inter-band energy levels therefore influence many solid state properties such as conductivity, luminescence, chemical reactions, corrosion or optical absorption. The presence of the defects will be equally apparent in mechanical properties or in phase transitions.

Insulators have large energy gaps so are transparent to visible radiation. Optical transitions of either type *A* or *B* of Fig 1 will produce optical absorption at the resonance energy, however, in a solid this is not a line absorption process as there may be coupling to the phonon spectrum. Consequently the absorption lines are broadened and the crystal may visibly change colour, hence the term colour centres to describe defect levels in insulators. Natural examples are ruby and sapphire which are the same host lattice (Al_2O_3) with impurity ions of either chromium or titanium. The same principle underlines the performance

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Table 1 Some typical defects in solids

Vacancies	Atoms missing from lattice sites. A variety of charge states may exist in insulating materials.
Interstitials	Atoms situated in between normal lattice sites
Impurities	All possible types and configurations
Clusters, precipitates	Either planar or three dimensional, in the same or new crystal phases.
Dislocations	Faults in the order of crystal planes.
Voids	Large aggregates of vacancies.
Bubbles	Vacancy clusters with inclusion of gas, for example from α particles.

of stained glass or colour filters which make use of optical absorptions bands ranging from the infrared to the ultraviolet. We should appreciate that the term "colour centre physics" is artificial as electronic transitions such as *B* or *C* of Fig. 1 may be described as photoconductive or luminescence phenomena.

The techniques for sensing the defects are often very sensitive and where it is possible to use electron spin resonance (ESR) or electron nuclear double resonance (ENDOR) the interactions with crystalline field and nuclear spins provide detailed information on the imperfection. Even if the defect does not contain an unpaired spin it is possible to follow changes in charge state and investigate thermal instability and defect interactions by the dispersive method of optical absorption or luminescence spectroscopy. With techniques available for detecting defects in parts per 10^{10} of normal atomic sites, all materials reveal departures from chemical purity The impurity level in most electronic devices is, however, irrelevant if the impurity ions do not take part in the process for which the device is designed. Therefore the approach is often to purify as far as possible, and then add a high concentration of known impurities to overshadow the residual effects of trace impurities. This can achieve a limited practical objective but may not, of course, increase our understanding of the role of defects, nor allow us to proceed to the manufacture of a similar device.

Classical colour centre studies have concentrated on intrinsic defects in the alkali halides. The reasons for this are partly historical It was possible to obtain single crystals with a simple structure, a wide band gap and visual effects of dissociation from exposure to X rays. Furthermore, the size of the defects is a function of the lattice parameter so that the transition energies scale with the sequence of alkali halides[7]. The most famous example, the F centre (an electron trapped at a vacant halogen lattice site), follows this pattern, and was positively identified by ESR and ENDOR. It is also a classic example of an electron in a box and by solving the simplest of Schrödinger equations we can predict the position of the optical absorption band. At this point it would be nice to say that the F centre is typical of the other intrinsic defects in alkali halides. In reality we have only established defect models for half a dozen centres in spite of forty years of worldwide research, and with hindsight much of the literature is valueless because of problems with impurities. Nor does this apply only to work with alkali halides or the results published in the distant past. Rather, it is exceptional to study a colour centre problem which is not qualified by statements about unknown imperfections. Alkali halide research happens to have reached a stage at which we can sense the weaknesses in the data but few other materials are so well understood. The only universal cure is to raise the art of crystal growing to a status at which it will attract the most able and dedicated scientists. All too often this phase of a research programme is left to someone else because it is quicker to produce "new" results from available material. (Maybe I should add that I too am guilty of this attitude) On the occasions on which I have consulted crystal growing manufacturers they have been helpful but for financial reasons do not wish to develop material for an uncritical market.

Imperfections and practical devices

We shall now look in more detail at three classes of device which exploit imperfections. The commonest depend on impurities. Intrinsic defects or modifications by radiation damage have only received serious attention since the advent of nuclear reactors. Frequently the impurities are added to charge-compensate other imperfections, as with semiconductors. For an optical example one may cite the case of fused silica which has useful properties in both the infrared and the ultraviolet. Here the presence of OH radicals introduces an infrared absorption band but produces the benefit that the OH also removes the absorption in the near ultraviolet region. Manufacturers of paints apply the same principle to produce whiter paints. A standard problem is a paint which contains titanium dioxide, which forms a range of compounds from TiO_2 to Ti. A deficiency of oxygen produces a yellow product as the band gap is effectively narrowed Charge compensation by ions of lower valency for example Al restores the transparency to the material by sharpening the optical absorption edge and removing the discoloration.

To digress a moment; we tend to expect compounds to obey Dalton's laws of gas composition and are surprised by materials like TiO_x $(0<x\leq 2)$. Dalton's laws need not apply in solids and it is quite possible to construct a lattice with an ordered array of vacant lattice sites. It is only our pre-

Table 2 Devices and fields of study which are dominated by the role of imperfections

Impurity related	Impurity plus radiation damage	Radiation damage
Semiconductors Phosphorescence Luminescence Photoemission Photoconductivity Solid state lasers Coloured gem stones Optical filters Optical windows Stained glass Paints	Corrosion Sensitivity of explosives Photographic process Photochromic glass Photolithography Radiation dosimeters Ion implanted semiconductors Ion implanted optical waveguides	Academic studies of colour centres Dark trace oscilloscopes Information storage Photolysis

Imperfections may be introduced as chemical impurities, by displacement of atoms from their normal lattice sites (radiation damage), or by a combination of the damage and impurity effects. The three columns provide examples of each.

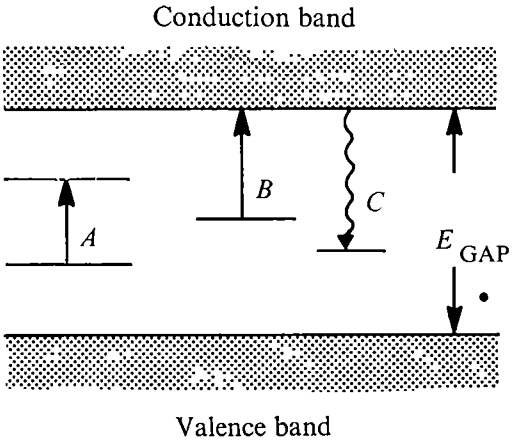

Fig. 1 The energy level diagram of an insulator which has localised imperfections Transitions *A* or *B* correspond to optical absorption, *C* is a radiative transition.

judice that describes these as imperfections. For this we suffer, as we then express the composition in rational numbers (for example $W_{50}O_{148}$) and are further surprised when such compounds have a simple unit cell.

Charge compensation can be reversed, for example by light which ionises one defect and transfers the charge to a second site. Consequently the colours of many paints and dyes are unstable in sunlight. Such materials are termed photochromic[8] The positive advantages of the light sensitivity are in photochromic glasses which darken in sunlight, or in information storage systems. Figure 2 outlines a suitable scheme of energy levels for an erasable information store. To write the data an energy *W* excites (writes) an electron from level (i) and transfers it to a different defect. The population of the ground state of (ii) is read by an energy *R* and erasure is achieved with *E*. There are many variations to this scheme. One example is $SrTiO_3$ doped with Mo^{6+} and Fe^{3+} on Titanium sites[9]. The charge transfer involves conversion of $Fe^{3+}\rightarrow Fe^{4+}$ and $Mo^{6+}\rightarrow Mo^{5+}$.

Thermal erasure is an alternative which is used in photochromic windows and "automatic" sunglasses. Again there is a wide choice of glass and impurity but at least one commercial material is based on small islands (<10 nm diameter) of AgCl in the glass matrix. The rationale for this is clear: AgCl has an optical absorption band in the near ultraviolet which initiates the photographic process. It is less obvious that it also has the advantage of being reversible. For this is not merely a charge transfer but decomposition of the lattice. The Ag^+ ions convert to Ag^0 ions which are no longer bonded to migrate through the lattice to form the latent image as specks of colloidal silver for example Ag_i^0. In the photochromic glass the decay product of the AgCl cannot leave the island of AgCl and enter the glass matrix which is in contrast with the reaction of Cl from AgCl with the gelatin of photographic emulsions. The close presence of free Ag and Cl in the glass example means the process is reversible.

The current view of the photographic process is slightly more complex than this, however, and requires the addition of impurity atoms and a change in chemical stability of grains with latent images within them. In broader terms the photographic process embodies all the principal kinds of imperfection in solids To summarise they are: (1) An initial charge transfer and chemical stability controlled by impurities. (2) Intrinsic defects produced by neutron, proton or other kinds of radiation and which react with themselves and existing imperfections. (3) The larger defects which cluster or precipitate out of the lattice tend to be more stable than point defects. These all lead to a modified crystal with an overall chemical stability which differs from the original sample.

An understanding of these problems opens new avenues of research for areas as apparently diverse as photolysis, corrosion, solubility, or catalysis as well as the formal solid state studies. Imperfections may increase or reduce chemical stability and photolysis. Among the more spectacular examples it has been found that several compounds undergo photolysis, explosively, when the impurity is reduced. Conversely radiation damage can enhance solubility. Here examples range from the conversion of an opal glass to a lithium metasilicate by photons, the use of photon and electron beams in lithography for miniature surface devices and changes in chemical solubility brought about by ion implantation. In all three cases, the solubility can be changed so much that the patterned area can be etched with negligible attack to the remaining areas. Each case however provides us with a different conceptual approach The metasilicate case involves a chemical phase change; in the lithographic example, the electrons produce ionisation and local rebonding which may also imply isolated point defects, whereas the ion implantation provides disordered regions along the ion tracks and very high concentrations of displaced atoms. The injection of ions at high velocity may also prove useful in changing the chemical imperfection properties of the solid as is evidenced by its widespread use in the manufacture of integrated electronic circuits. A related field of optical circuitry of surface waveguides, acoustic wave devices, modulators and couplers is now developing and also links together the problems, and advantages, of colour centres and ion implantation[10-12]. These examples seem to have brought us up to the present state of the art and I shall conclude with a tentative look at a future problem.

Colour centres and fusion reactors

Fission reactors produce a range of fission fragments with a wide spectrum of energy and mass which cause damage in the structural materials. In general terms the radiation damage is to be expected and to some extent is allowed for in the reactor design, either by the choice of materials which are relatively unchanged or, in extreme cases, by allowing for replacement sections. With each new reactor, however, new problems arise because of the higher operating temperatures, higher fluxes or reactivity of the coolants.

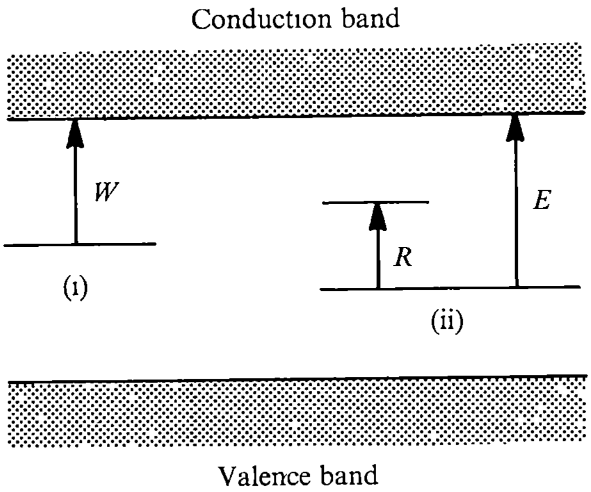

Fig. 2 An energy level scheme which would be suitable for information storage Writing is performed by transfer of an electron from defect (i) to defect (ii). Read measurements are made by sensing the population of the defect (ii) ground state by the optical transition *R*. Erasure proceeds by transfer to defect (i) by energy *E*. In this example all three optical processes are in distinct energy regions.

Many of these problems will also exist in fusion reactors and an obvious case to consider is what would happen if the fusion events are triggered by pulses of laser light. Laser beams deposit energy in a glass by absorption or scattering. If the pulses of light are sufficiently intense then the rate of energy deposition will cause the glass to explode[13]. For this reason one is very careful to remove strain or refractive index gradients from the glass and in normal environments the main concern is with the polish of the surface layer. Mechanical polishing leaves residual strains which may set an upper limit to the power density before fragments of material explode from the surface Chemical or ion beam etching of this surface strain can raise the threshold level from 2 to 10 GW cm^{-2}.

In a radioactive environment fission tracks, colour centres or large scale aggregates of defects can all produce the same catastrophic events in the bulk of the specimen. Further, because the damage is unlikely to be uniform through the material there is also mechanical strain. In early fission reactors the solution was to heat the materials and anneal the damage. In later reactors this has not always been possible because of the risk of enhancing the formation of large stable defects such as voids or gas bubbles. The research on voids has also indicated that they are produced at a higher rate at high fluxes (this is the situation in a pulsed fusion system). In short, we shall need glass windows, and possibly laser materials, which are in close proximity to the fusion vessel which must not (1) distort (2) colour (3) accrete large scale defects or (4) increase the surface scatter. Problems (1), (2) and 3 are fairly intractable, (4) is certainly insoluble because sputtering of surface atoms is a consequence of radiation damage. In an insulating material the problem can be further aggravated by charge build up below the surface followed by explosions in which blocks of material are ejected. This would cause laser scatter and destruction of the glass by the subsequent laser pulses.

Clearly with a little thought we could find other potential problems. Instead, I would suggest these problems are not insurmountable. For example the oxide glasses with a large inherent vacancy concentration (for example TiO_2) are essentially self-annealing and remarkably stable to radiation damage. Similarly, if this type of material could be made of high enough electrical conductivity, yet remain transparent, then the problems of surface charge explosions might also be avoided.

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articles

Linkage disequilibrium between *H–2* and *t* complexes in chromosome 17 of the mouse

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Mice with t *factors belonging to the same complementation group carry similar, if not identical,* H–2 *haplotypes although these factors were derived from widely separated geographical areas. This association between* t *and* H–2 *complexes suggests more than a casual relationship between the two complexes, at least at the population level.*

CHROMOSOME 17 of the mouse carries two of the most puzzling systems in mammalian genetics, *H–2* and *t* (refs 1–3). The question whether the presence of two such highly sophisticated systems on the same chromosome is fortuitous or whether the systems could be genetically interrelated has been raised repeatedly[2–6]. In this communication we demonstrate at least one level of interrelation between *t* and *H–2* by showing the presence of a strong linkage disequilibrium in the mouse population between the two complexes.

While studying the polymorphism of serologically detectable H–2 antigens among wild mice[7] we H–2 typed strains carrying various *t* factors because most of these factors have been extracted from wild mouse populations, and there was a good chance that they carried the wild-derived *H–2* haplotypes. The *t* bearing strains were from our mouse colony or that of Dr Dorothea Bennett at Cornell University Medical College. The former were strains at various stages of transfer of individual *t* factors (originally obtained from the late Professor L. C. Dunn) on the inbred background of strain C57BL/10Sn (B10); the

Table 1 H–2 antigens in *t*-bearing strains of mice.

	H–2	H–2 antigens																		Ia antigens
Factor	haplotype	1	2	3	4	5	6	7	8	11	13	23	25	31	105	106	107	108	109	101
t^{12}	*t12*	—	—	—	—	5	—	—	—	—	—	—	—	—	—	106	107	—	—	.
t^{w32}	*t12*	—	—	—	—	5	—	.	.	—	—	—	—	—	—	106	107	—	—	—
t^{w2}	*w5*	—	—	"3"	—	—	"6"	C	8	—	—	—	—	31	105	—	107	—	—	—
t^{w8}	*w5*	—	—	"3"	—	—	?	.	.	—	.	—	—	31	105	—	107	—	—	—
t^{0}	*w5*	—	—	"3"	—	—	?	.	.	—	.	—	—	31	105	—	107	—	—	—
t^{1}	*w5*	—	—	"3"	—	—	?	.	.	—	.	—	—	31	105	—	107	—	—	—
t^{6}	*tw1*	—	—	—	—	C	—	—	—	—	"13"	—	—	—	—	—	—	108	—	—
t^{w1}	*tw1*	—	—	—	—	C	—	—	—	—	"13"	—	—	—	—	—	—	108	—	—
t^{w71}	*tw1*	—	—	—	—	—	—	.	.	—	.	—	—	—	—	—	—	108	—	—
t^{w73}	*tw1*	—	—	—	—	?	—	.	.	?	.	—	—	—	—	—	—	108	—	—
t^{w12}	*tw1*	—	—	—	—	?	—	.	.	?	.	—	—	—	.	—	—	108	—	—
t^{w5}	*tw5*	"1"	—	—	—	5	—	—	—	11	—	C	25	—	—	—	—	—	109	101
t^{w75}	*tw5*	—	—	—	—	?	—	.	.	11	.	—	25	—	—	—	—	—	109	—

(Antigens H–2.2, 4, 9, 15, 16, 17, 18, 19, 28, 30, 32, and 33 were found to be absent in all tested t strains.)
A dot indicates not done. Quote marks indicate a similar but not identical antigen. C, cross-reacting antigen. A question mark indicates that presence or absence of an antigen is uncertain.

latter were mostly outbred animals maintained as balanced lethal lines in combination with the *brachyury* chromosome (*T*/*t*). The detailed results of this typing will be presented elsewhere; here we summarise the data and demonstrate a correlation between *t* complementation groups and *H–2* haplotypes.

The first step in H–2 typing was to use cells from t strains as targets for haemagglutinins and/or cytotoxins produced against known H–2 antigens. This provided limited information about the t strains, since few positive reactions were observed. Most known H–2 antigens were absent in the t strains; however, those present had a distribution more or less coinciding with the classification of *t* factors into complementation groups (antigens 1–31 in Table 1). Thus, t^{12} and t^{w32}, two members of the t^{12} group, both carry H–2.5 and t^{w5} and t^{w75}, two members of the t^{w5} group, carry antigens H–2.11 and H–2.25. In addition, t^{w1}, t^{w71} and t^{w12}, all members of the t^{w1} group and t^{w73}, which seems to define a new complementation group[13] also lack most known H–2 antigens. The two semi-lethal factors, t^{w2} and t^{w8} have a similar antigenic pattern. But the factors belonging to the t^{0}group, t^{0}, t^{1} and t^{6}, possess different antigenic patterns.

Because most known H–2 antigens were absent in t strains, we tested whether these strains carry new, so far unidentified, H–2 antigens. We immunised inbred strains and their hybrids with tissues from *t*/+ heterozygotes sharing an *H–2* haplotype (the one present in the + chromosome) with the recipient. We produced five antisera (Table 2) which were particularly useful in characterisation of t strains. Analysis of these antisera led to a definition of five new antigens, shown by linkage tests to be controlled by genes closely linked to *H–2* (data not shown). Antigens 101 and 106 to 109 were absent in all inbred strains, and H–2.106, 107 and 108 apparently occurred with a relatively low frequency among wild mice[24]. As Table 1 shows, however, the antigens are shared by some of the t strains, and this sharing, to a certain degree, parallels the classification of *t* factors into complementation groups.

One possible explanation of this peculiar antigenic similarity among members of the same complementation group is that our antisera detect not H–2 antigens, but products of the *t* loci. To test the relationship between the *t* factors and the new antigens, we first established the tissue distribution of the antigens. As Table 3 shows, the distribution of H–2.106, H–2.107 and H–2.108 is that of classical H–2 antigens since reactivity against antigens was cleared or reduced by spleen, lymph nodes, thymus, liver, kidney, heart, brain and erythrocytes. In contrast the antigen detected by the cytotoxic reactivity of H–27 is present only on spleen and lymph node cells, and the antiserum reacts only with bone-marrow-derived (B) lymphocytes and not thymus-derived (T) lymphocytes (Fig. 1). The tissue distribution of this antigen is thus indicative of an Ia antigen[8], and this antigen has been designated Ia.101. H–27 also has reactivity in the PVP-haemagglutination test in which it detects H–2.109. As this reactivity is weak, however, the assignment of H–2.109 should be considered tentative.

We also tested the molecular relationship of the new antigens to the few known H–2 antigens demonstrable in t strains. This test was based on the technique monitoring redistribution in the cell membrane of antigens combined with two layers of antibodies[9]. A typical experiment of this sort is shown in Table 4. In this experiment, pretreatment of $+H\text{–}2^{d}/t^{12}H\text{–}2t^{12}$ spleen cells with K–46 (anti-H–2.5) induced resistance to H–12 (anti-H–2.106) but not to the anti-H–2^{d} sera, K–304 (anti-H–2.4) and K–25 (anti-H–2.31) and vice versa. It thus seems that H–2.5 and H–2.106 are on the same molecule, as they are redistributed in the cell membrane together.

On the basis of these data, we conclude that antigens H–2.106 to H–2.109 are not the products of the *t* loci but typical H–2 antigens; 101 seems to be an Ia antigen. The presence of the new antigens on adult somatic tissues makes it unlikely that they are products of *t* loci, which are expressed only during embryogenesis and on spermatozoa[10].

If one accepts this conclusion, members of the same complementation group of *t* factors must have either identical or very

Table 2 Antisera detecting H–2.106, H–2.107, H–2.108, H–2.109 and Ia 101

Code No.	Recipient	Donor	*H–2* haplotype combination†	Antigens detected
H–12	(B10×A)F$_1$	(B10×*T*/t^{12})NT‡	(*b*/*a*)*b*/*t12*	H–2.106, H–2.107
H–25	(B10.D2×D2.GD)F$_1$	t^{w2}/t^{w2}	(*d*/*g2*)*tw5*/*tw5*	H–2.107
H–11	(B10×A)F$_1$	(B10×*T*/t^{w1})NT	(*b*/*a*)*b*/*tw1*	H–2.108
H–26	(B10×A)F$_1$	(B10×*T*/t^{6})NT	(*b*/*a*)*b*/*tw1*	H–2.108
H–27	(B10×C3H)F$_1$	(B10×*T*/t^{w5})NT	(*b*/*k*)*b*/*tw5*	H–2.109, Ia.101

† Recipient in parentheses.
‡ Normal tail.

similar alleles at the loci coding for serologically detectable H-2 antigens. Our preliminary data on mixed lymphocyte reaction typing of t strains parallel the serological data and thus suggest that the intra-group similarity encompasses a large portion of the *H-2* complex. The significance of this finding becomes apparent when one takes into account the origin of the individual *t* factors. For example, t^{w1} was extracted before 1955, from a wild mouse captured in the New York area[11]; t^{w71} was obtained from a wild mouse trapped in 1968 in Denmark[12]; and t^{w12} was derived from a wild mouse captured in California before 1956 (ref. 13). All three factors belong to the same complementation group and all carry *H-2* haplotypes characterised by the presence of antigen H-2.108. Thus, individual members of the same complementation group are unrelated in terms of place and time of their extraction from wild mouse population, but they have very similar *H-2* haplotypes. Similar relationships exist among members of other complementation groups. The only serious exception is the t^0 group: the t^6 factor which is classified as a member of the t^0 group, has an *H-2* haplotype which resembles that of the t^{w1} group, while t^0 and t^1 have haplotypes similar to $H\text{–}2^{w5}$. But, t^0, t^1, and t^6 are among the factors maintained for the longest time in the laboratory, and therefore they might have been modified by recombination. Supporting the contention is the fact that, for example, t^6 has a relatively low transmission ratio (0.67 C. H., unpublished). (Factors extracted from wild mice usually have transmission ratios of > 0.9; factors derived by recombination from t^{wild} factors usually have much smaller ratios.) It is possible that t^6 is a recombinational derivative of another factor (t^{w1}), while $t^0(t^1)$ or a *t* factor from which t^0 is derived may be responsible for the dispersal of the $H\text{–}2^{w5}$ haplotype among wild mice.

Table 3 Tissue distribution of H-2.106, H-2.107, H-2.108, and Ia.101

	Reciprocal of titre after absorption with the following tissues*															
	Spleen		Lymph nodes		Thymus		Liver		Kidney		Heart		Brain		Erythrocytes	
Antigen	t strain†	B10	t strain	B10	t strain	B10	t strain	B10	t strain	B10	t strain	B10	t strain	B10	t strain	B10
H-2.106‡	—	128	—	128	—	128	—	128	—	128	—	128	—	128	—	128
H-2.107§	—	960	—	960	—	960	—	1,920	—	1,920	120	960	240	960	—	960
H-2.108 ¶	—	512	—	512	128	512	—	512	128	512	266	512	256	512	256	512
Ia.101 ‖	—	8	—	16	8	8	16	16	8	16	16	16	16	16	8	16

* Absorption was done with one part packed tissue: two parts diluted antiserum for 30 min at room temperature, then 30 min at 37 °C.

† Tissue used for absorption was from the strain against which the absorbed serum was then tested.

‡ H-12 was diluted 1:64, absorbed, then tested against $+/t^{12}$ lymphocytes in a cytotoxic test[8] (an unpublished work with K. Artzt and D. Bennett); titre was the lowest dilution giving 50% cell lysis.

§ H-25 was absorbed first with B10.BR, then diluted 1:10 and absorbed with the above tissues, and tested against t^{w2}/t^{w2} erythrocytes in a PVP-haemagglutination test[24]; titre was the lowest dilution giving an agglutination score of 1.

¶ H-11 was diluted 1:64, absorbed, then tested against $+/t^{w1}$ lymphocytes in a cytotoxic test; titre was the lowest dilution giving 50% cell lysis.

‖ H-27 was diluted 1:2, absorbed, then tested against $+/t^{w5}$ lymphocytes in a cytotoxic test; titre was the lowest dilution giving 50% cell lysis.

The finding that *t* chromosomes within the same complementation group carry similar *H-2* haplotypes has important implications with respect to the origin of *t* factors and their role in maintaining *H-2* polymorphism. There is a wealth of experimental data indicating that wild mice live in small, relatively isolated units or demes with a social structure dominated by a single male (review in ref. 1). Because of the lethality of *t* factors, these factors can never become fixed in any particular deme and the *t* factors already present in a deme are eliminated by random genetic drift, and new factors are reintroduced into the demes[14]. The question then arises: what is the source of the reinfecting *t* factors? Do the factors represent new mutations (in the broadest sense of the word) or are the same factors reintroduced over and over by interdemic migration? Our data strongly suggest that the latter of the two explanations is correct. It seems that there is only a limited number of different *t* chromosomes among wild mice (perhaps fewer than there are complementation groups) and that these chromosomes have been perpetuated in mouse populations for a very long time.

This interpretation of *t* population behaviour suggests a far greater role for interdemic migration that was originally postulated. Demes have been believed to be virtually impenetrable to migrants and the migration rates were thought to be extremely low[15]. If the only source of *t* factors is, however, immigration, then the migration rates must be quite significant.

Our data also have an important implication concerning the relationship between the *t* and *H-2* systems. The data indicate that, at the population level, the two systems are interrelated in that certain *t* factors are associated with specific *H-2* haplotypes. In other words, the *t* factors and *H-2* haplotypes show a strong linkage disequilibrium. What could be the significance of this disequilibrium? The simplest interpretation is that the disequilibrium is the consequence of the *de facto* close linkage of the two complexes (W. Bodmer, personal communication). As discussed by Thomson *et al*[16], linkage disequilibrium between two closely linked loci can persist over long periods if a relatively small selective effect is acting on one of these loci. For example, in a typical Northern European human population, the frequency of the *HLA1* allele is 0.16 and of the *HLA8* is 0.13. The frequency of the *HLA1*, 8 haplotype is 0.08, which corresponds to linkage disequilibrium $D = 0.06$. At the same time, the distribution of this haplotype throughout Europe suggests that it has been present at reasonable frequencies since pre-agricultural times. The association between certain *t* factors and specific *H-2* haplotypes could be an analogous example of a presistent linkage disequilibrium.

This interpretation, however, does not explain the nature of the selective effect maintaining the linkage disequilibrium. In contrast to disequilibrium in the *HLA* system, where selection acts on loci that are physically close to each other on the chromosome and where the haplotypes that are in disequilibrium show no evidence of crossing over suppression, an active suppression is a vital part of the mechanism maintaining the *t–H-2* disequilibria. Furthermore, to achieve this suppression, the mouse apparently pays a high price in the form of homozygous lethality of the embryos. One would therefore expect that there is a good reason for this costly invention. What might this reason be? We propose that the selective advantage of the linkage disequilibrium lies in the production of genetically superior sperm. It is known that the loci of the *t* complex are expressed on sperm and that they affect sperm behaviour[17]. The sperm carrying a *t* mutation has a much higher probability of fertilising an egg than the normal or the *T* sperm and the consequence of this effect is the distorted segregation observed in a $t/+ \times +/+$ mating. Circumstantial evidence indicates that a group of loci between *T* and *H-2* in chromosome 17 influences the physiological properties of the sperm[18,19]. One can therefore envision that certain combinations of alleles at these loci result in a physiologically superior sperm, and that, for this reason, these combinations are selected for in natural populations of the mouse. When a structural aberration suppressing crossing over occurs in the chromosome carrying an advantageous combination of alleles, the alleles are permanently locked together and the chromosomal segment transmitted intact from generation to generation. Theoretically, such an advantageous combination should spread rapidly throughout the population and replace all less advantageous combinations of alleles. In reality, however, this does not happen because the chromosomal aberration also has a deleterious effect on its bearer in that it causes homozygous lethality of the embryos and the lethal effect then balances out the advantageous sperm effect. This interplay of the two opposing forces is possible because of the subdivision of wild populations into demes in which genetic drift[15] and inbreeding[20] keep the frequency of the *t* factors below deterministic expectations[21]. The result of the interplay is that the interlocked combination of alleles (a particular *t* factor) is maintained in the population in spite of the deleterious effect, but because of this effect it is prevented from completely taking over the population. Consequently, a particular *t* factor persists in the population with a stable frequency determined by the interaction between the two opposing forces, segregation distortion, and lethality. In cases where the crossing over suppression effect is strong enough to encompass the *H–2* complex, a particular *H–2* haplotype is locked together with a particular *t* factor, and a linkage disequilibrium of this combination then develops. In the history of the mouse population the locking together of the alleles occurred independently several times and involved different allelic combinations of parts of chromosome 17 and different *H–2* haplotypes.

According to this concept most complementation groups are the result of independent occurrences of the locking together event. On the other hand, the various *t* factors of the same complementation group are all derived from the same locking together event, although they might have subsequently differentiated by mutation. Some members of a complementation group, however, might have been derived from a *t* factor of a different complementation group by a rare recombination within the *t* complex.

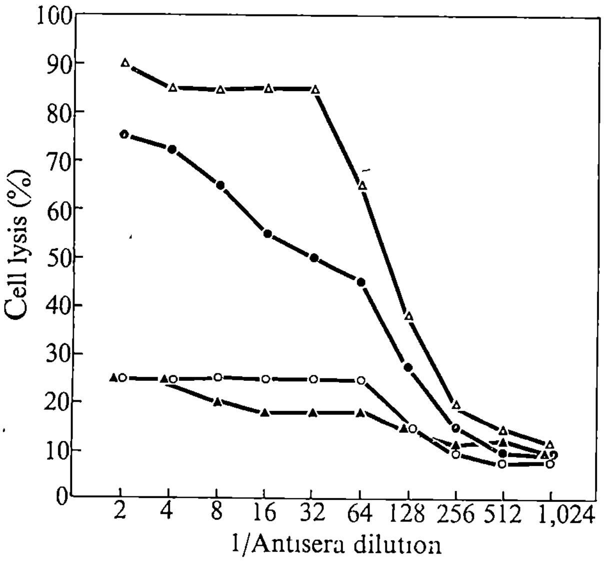

Fig. 1 Cytotoxic reactivity of anti-Ia.101 and anti-Thy-1.2 (θ-C3H) sera with thymus-derived lymphocytes (T cells) and bone-marrow-derived lymphocytes (B cells) from t^{w5} spleen. T cells were obtained by filtration of spleen cells through nylon fibre columns[23]. B cells were prepared by the method of Hauptfeld *et al.*[8]. The cytotoxicity test was performed as described by Hammerberg *et al.*[8] (unpublished). ○, anti-Thy-1.2 reactivity against B cells from t^{w5} spleen; ●, anti-Ia.101 reactivity against B cells from t^{w5} spleen; △, anti-Thy-1.2 reactivity against T cells from t^{w5} spleen; ▲, anti-Ia.101 reactivity against T cells from t^{w5} spleen.

Table 4 Susceptibility of $+H\text{–}2^d/t^{12}H\text{–}2^{t12}$ spleen cells to cytotoxic killing after anti-*H–2* and GAMIG treatment

Pretreated with*	Tested with	Dead cells at dilution of antiserum (%) 1:2	1:4	1:8
GAMIG (1:4)	H–12	88	85	80
H–12 + GAMIG (1:4)	H–12	25	25	25
K–46† + GAMIG (1:4)	H–12	38	30	30
K–304‡ + GAMIG (1:4)	H–12	85	82	80
K–25§ + GAMIG (1:4)	H–12	88	80	72
GAMIG (1:4)	K–46	70	60	50
H–12 + GAMIG (1:4)	K–46	30	35	35
K–46 + GAMIG (1:4)	K–46	25	25	20
K–304 + GAMIG (1:4)	K–46	70	65	60
K–25 + GAMIG (1:4)	K–46	65	60	50
GAMIG (1:4)	K–304	90	90	90
H–12 + GAMIG (1:4)	K–304	88	85	80
K–46 + GAMIG (1:4)	K–304	85	85	80
K–304 + GAMIG (1:4)	K–304	25	25	25
K–25 + GAMIG (1:4)	K–304	85	85	80
GAMIG (1:4)	K–25	85	85	80
H–12 + GAMIG (1:4)	K–25	85	80	80
K–46 + GAMIG (1:4)	K–25	85	80	80
K–304 + GAMIG (1:4)	K–25	85	85	80
K–25 + GAMIG (1:4)	K–25	25	25	20

The cytotoxicity test was performed as described by Hauptfeld *et al* (ref. 8). GAMIG, goat anti-mouse Ig (γ,κ), was produced and supplied by Dr E. S. Vitetta, University of Texas. Haplotype *H–2*^{d} possesses H–2 4 and H–2 31.

* All anti-H–2 sera were used undiluted. Cells pretreated with GAMIG and tested with normal mouse serum and complement had a background of 15% cell lysis.

† K–46: (HTG × B10 D2)F$_1$ anti-B10; anti-H–2 5.

‡ K–304: (A BY × B10.AKM)F$_1$ anti-B10.A; anti-H–2.4.

§ K–25: (A × B10.A)F$_1$ anti-B10.D2; anti-H–2.31.

Whether the *H–2* complex has any evolutionary homology to the *t* complex is not known. But, the finding that antigens coded for by one of the *t* loci have a similar molecular weight as the classical H–2 antigens[22] suggests that such homology might indeed exist. In any event, it is clear that the *H–2* complex, at least at the population level, is included in the sphere of action of *t* and thus is a part of the *t* supersystem.

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Evidence for cell surface control of macronuclear DNA synthesis in *Stentor*

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In cell grafts, Stentor *macronuclei associated with separate regions of cell surface can be made asynchronous with regard to morphology and DNA synthesis even though they demonstrably share a common endoplasm. These results suggest a mechanism for nuclear differentiation within a single cytoplasmic compartment, based on cell surface differences.*

THE baffling phenomenon of nuclear differentiation within a single cytoplasmic compartment occurs in many animal and plant cell types (for example, developing eggs[1], grasshopper neuroblast cells[2], pollen grains[3], cells of the developing stomatal complex[3], ciliates[4] and foraminiferans[5]). In most ciliates, nuclear differentiation occurs during the formation of micro- and macronuclei from a single zygote nucleus during sexual reproduction (conjugation). The two types of nucleus, which differ strikingly in morphology and nucleic acid content, develop in different regions of the cell and centrifugation experiments have shown that in *Tetrahymena*[6] and *Paramecium*[7] the developmental fate of the presumptive nuclei can be altered by displacing them.

Here I report some experiments with *Stentor* which suggest a possible mechanism for the generation of such nuclear differences in morphology and synthesis. Previous experiments on the role of the cell surface in generating asynchrony of nuclear morphology[8,9] have now been extended; the results indicate that nuclei with asynchronous morphology are also asynchronous with respect to DNA synthesis.

Experimental system

Stentor is a large (1 mm) ciliate which is one of the classic organisms for studies involving cell microsurgery. The most prominent feature of cell division is the development of the oral primordium, which gives rise to the feeding structures of the posterior daughter cell by production of kinetosomes (basal bodies) and associated structures at the junction of wide and narrow striping on the ventral cortex. The stages of oral development have been assigned numbers 1–8 (ref. 10), with each stage lasting about 1 h at 20 °C. While the primordium is developing, the chain macronucleus first coalesces (stage 5) into a compact mass (stage 6), then elongates (stage 7) and renodulates so as to double the number of nuclear nodes in preparation for division. After a G_1 period of 2–12 h, macronuclear DNA synthesis is continuous throughout interphase (36–48 h) and stops at or near the time of nuclear coalescence[11]. Similar morphological and synthetic events of macronuclear replication also occur if oral development is induced during interphase by removing the feeding structures; they are more conveniently studied during this process (regeneration, Fig. 1) because of the ease with which large numbers of cells can be obtained in any given stage. My experiments therefore involved regenerating stentors.

A stentor in early regeneration continues to develop when grafted to a morphostatic cell of comparable size; when it reaches stage 6 and undergoes nuclear coalescence it induces this event in its graft partner as well[12]. Nuclear coalescence is, however, usually not induced if a thin, transverse disk taken from a third (morphostatic) stentor is interposed as a barrier between the two main graft components. This operation (Fig. 2) produces visible double rings of discontinuity in the striped cortical surface layer (Fig. 3) which heals slowly over a period of 3–4 h. Although the cortical membrane heals with time, breaks in the cortical fibrillar structures (kinetodesmal fibres, and myonemes) do not heal. When the food vacuoles of one graft component were labelled with small plastic spheres before the operation, the spheres became evenly distributed throughout the endoplasm of the entire graft complex within 10–15 min[8]; the two members of the graft complex therefore share a common endoplasm from the time of the operation. The fact that cortical discontinuities were usually accompanied by nuclear asynchrony although the endoplasms mix freely suggested that nuclear morphology might be controlled by cell surface changes rather than by diffusion of a regulatory substance through the endoplasm from the regenerating component to the morphostatic one. Since DNA synthesis normally stops at the time of macronuclear coalescence, it seemed

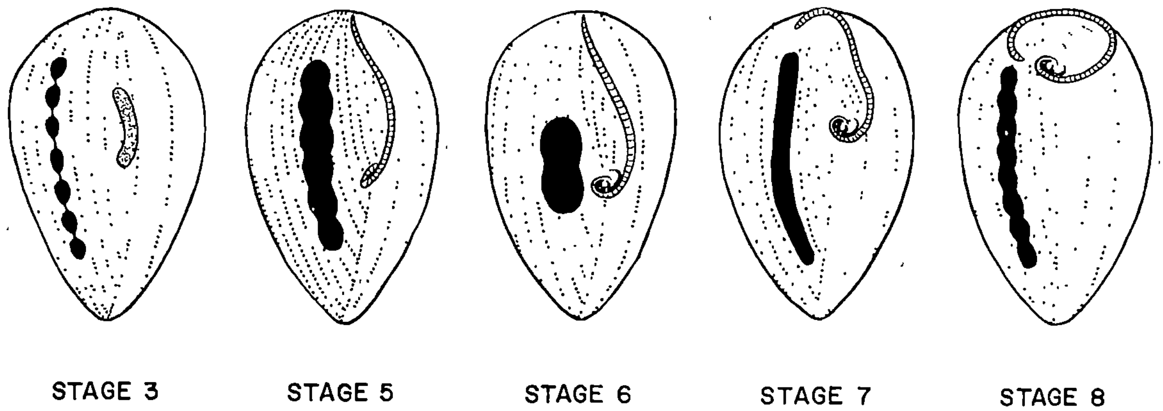


Fig. 1 Some stages of oral regeneration in *Stentor*. Stage 3, showing curved oral primordium (stippled); stage 5, nuclear coalescence; stage 6, gullet formation; stage 7, nuclear elongation; stage 8, nuclear nodulation.

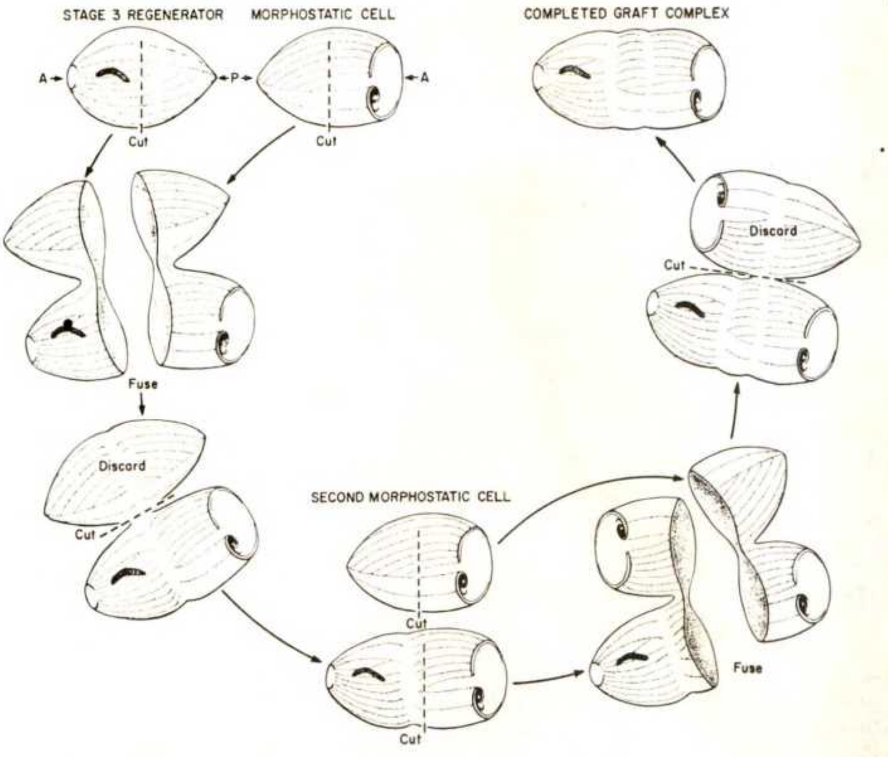


Fig. 2 Procedure used in making graft complexes. The anterior half of a cell in stage 3 of regeneration is grafted to a morphostatic stentor in heteropolar orientation. A cut is then made in the morphostatic stentor just below the line of heal and the anterior half of a second morphostatic cell is added in homopolar orientation with respect to the first one. Most of the first morphostatic cell is removed during the operation so that it persists only as a thin disk separating the graft components.

interesting to examine graft complexes with asynchronous nuclear morphology for gross asynchrony of DNA synthesis.

Induced asynchrony of macronuclear DNA synthesis

S. coeruleus (Stella strain, obtained from Dr Vance Tartar) was cultured in a modified Peters' solution; the food organism was *Tetrahymena pyriformis* grown on *Aerobacter aerogenes*. The cultures, kept in Petri dishes, were fed *ad libitum* from 1000 to 1800, starved overnight and used the following day. This schedule generally produced cultures that were well enough fed for most cells to be synthesising DNA but starved long enough to clear the endoplasm of most food vacuoles. This proved necessary to permit observation of nuclear morphology and facilitate microsurgery, which was carried out free-hand with a glass needle. DNA synthesis was assessed by transferring graft complexes to Peters' medium containing ^{3}H-thymidine (New England Nuclear or Amersham Searle, 17–20 μCi mmol^{-1}, 50 μCi ml^{-1}) for 30 min. Squash preparations were processed for autoradiography with Kodak NTB3 emulsion as described previously[11]. Regeneration was induced by a brief treatment with 10% sucrose, which removes the band of adoral membranelles from the oral apparatus and initiates oral primordium formation.

Preliminary studies confirmed previous work on dividing[11] and regenerating[13] stentors; silver grains were not found over the coalesced or elongating nuclei of regenerating stentors, although morphostatic cells and cells in earlier stages of regeneration taken from the same culture showed heavy nuclear label. The absence of label at stages 6 and 7 is not caused by failure of the cells to take up ^{3}H-thymidine since coalesced and elongating nuclei are unlabelled even when the isotope is injected[13].

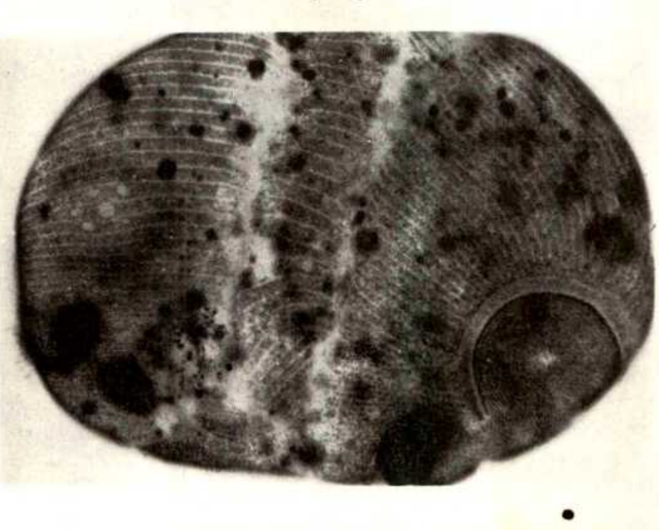
Graft complexes with regenerating (stage 3) and morphostatic components separated by cortical discontinuities were prepared in groups by grafting for 1 h; they were then maintained in Peters' medium until nuclear coalescence had occurred in the regenerating component 2–3 h later. They were transferred to Peters' medium containing ^{3}H-thymidine, squashed on slides in a known orientation and prepared for autoradiography. The resulting autoradiographs were examined for gross differences in labelling. The differences were so great that only a few graft complexes were difficult to evaluate visually and these were discarded along with unlabelled graft complexes.

Ninety-six per cent of the 52 graft complexes examined were grossly asynchronous with respect to DNA synthesis (Fig. 4*a*); a typical example is shown in Fig. 5. The chain of nodes was always more heavily labelled than the coalesced nucleus.

Nuclear coalescence was induced in some graft complexes with cortical discontinuities, presumably because cortical healing sometimes occurred rapidly enough to facilitate re-establishment of synchrony. In all of 39 such graft complexes examined, both coalesced nuclei were unlabelled (Fig. 4*b*).

To examine synchrony in graft complexes lacking cortical discontinuities, a large stentor in stage 1 of regeneration was grafted directly in heteropolar orientation to a morphostatic cell; the graft complexes were incubated in Peters' medium for 2–3 h, placed in ^{3}H-thymidine for 30 min and prepared for autoradiography. Synchrony of nuclear labelling was found in 99% of the 73 labelled graft complexes examined (Fig. 4*c*).

Fig. 3 Newly created graft complex, showing double rings of cortical discontinuity at graft boundaries.



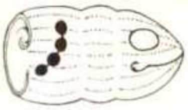
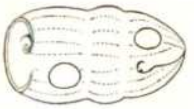
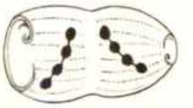
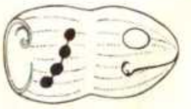
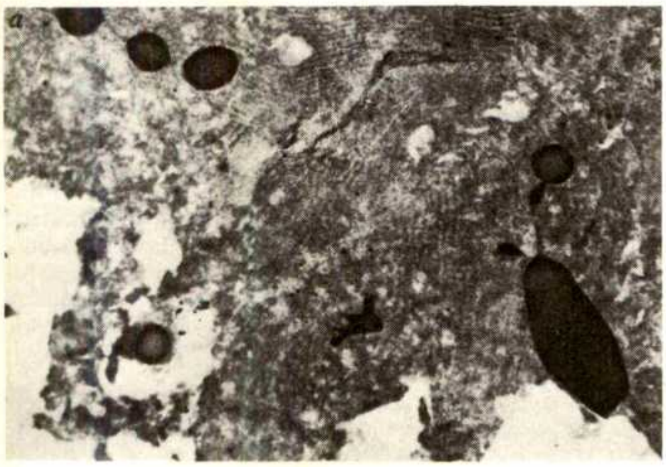
	TYPE OF GRAFT COMPLEX	TOTAL NO. OF GRAFT COMPLEXES	DNA SYNTHESIS IN GRAFT COMPLEXES	
			Synchronous	Asynchronous
a		52	2 (4%)	50(96%)
b		39	39 (100%)	—
c		73	72 (99%)	1(1%)
d		17	2 (12%)	15(88%)

Fig. 4 Summary of experiments on induced asynchrony of macronuclear DNA synthesis in *Stentor* graft complexes. *a*, Morphostatic stentor grafted to stentor in stage 3 of regeneration; graft components separated by cortical discontinuities. The regenerating component has reached stage 6 and nuclear morphology is asynchronous. *b*, Same as (*a*) except that nuclear morphology is synchronous presumably because of cortical healing. *c*, Morphostatic stentor grafted to stentor in stage 1 of regeneration; no cortical discontinuities. *d*, Morphostatic stentor grafted to stentor in stage 5; regenerating component has reached stage 6. Asynchronous nuclear morphology; no cortical discontinuities.

Since stentors selected at random from a given culture usually differ widely in extent of labelling, this strongly suggested that the graft complexes had become synchronised. Later experiments showed that a similar degree of synchrony occurred after 1 h in Peters' medium.

Since the central barrier ring does not interfere with the exchange of food vacuoles between the graft components, one would not expect it to impede the free exchange of ^{3}H-thymidine. But this was tested directly. *Stentor* takes up isotopes primarily through the gullet and decapitated organisms accumulate little or no label[14]. Graft complexes with cortical discontinuities were prepared and the oral apparatus was excised from one graft component at the time of the operation. After 2–3 h in Peters' medium, the graft complexes were placed in ^{3}H-thymidine for 30 min and squashed in a known orientation. If the discontinuities had interfered with diffusion of ^{3}H-thymidine, one would expect the nucleus of the intact component to be more densely labelled but examination of



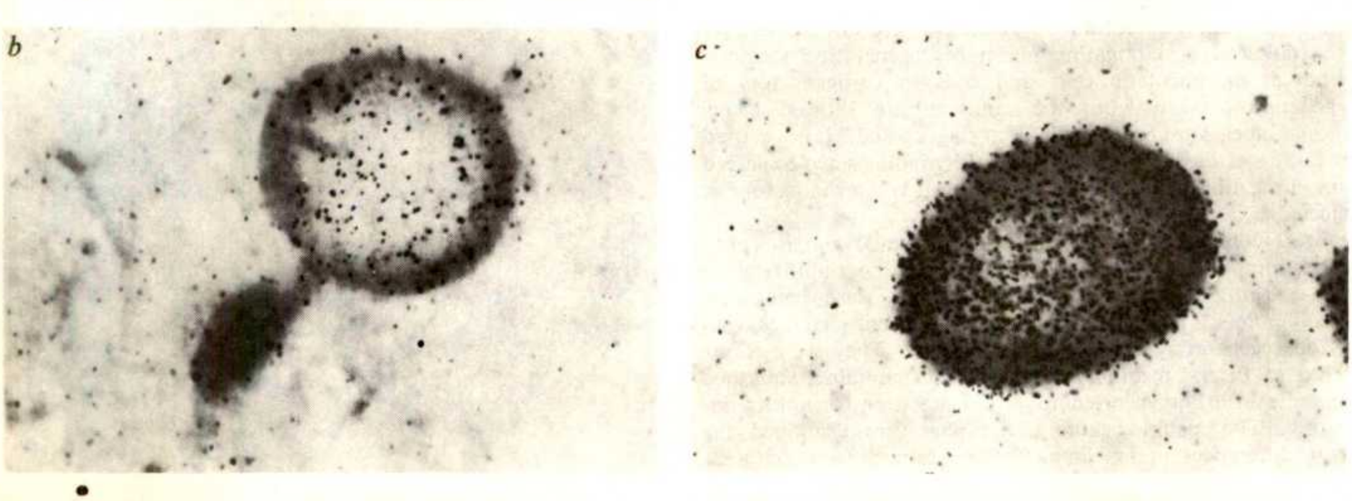


Fig. 5 Macronuclear DNA synthesis in graft complex with differential nuclear morphology. *a*, Graft complex, with coalesced nucleus of regenerating component (right) and three nuclear nodes of morphostatic component (left) (×140). *b*, Autoradiograph, part of the coalesced nucleus shown in (*a*) (×880). *c*, Autoradiograph, one node of the chain-form nucleus shown in (*a*) (×880).

32 such graft complexes showed that when differences in extent of labelling occurred they were random.

A final experiment was designed to determine whether the asynchrony of DNA synthesis in graft complexes with cortical discontinuities might be entirely or partly caused by the fact that one nucleus was in the chain form and the other was coalesced. This seemed likely because a coalesced nucleus transferred for 2–3 h to an S phase stentor resumes DNA synthesis but is less densely labelled than the chain nucleus of the host cell[7]. Coalesced nuclei, however, might behave differently in graft complexes containing their own cytoplasm as well as interphase cytoplasm; DNA synthesis was therefore examined in graft complexes containing half of a morphostatic stentor and half of a stentor in stage 5 or 6. In such grafts, the nucleus of the morphostatic component is not induced to coalesce but persists as a chain of nodes while the nucleus of the differentiating component is coalesced. Groups of such graft complexes, prepared over a 30-min period, were incubated in Peters' medium for 1 or 2 h, transferred to ^{3}H-thymidine for 30 min and prepared for autoradiography. In 59% of the 17 labelled graft complexes examined, the coalesced nucleus was labelled, indicating that coalesced nuclei can be induced to resume DNA synthesis when they are part of a stage 6-morphostatic graft. Eighty-eight per cent of the graft complexes showed gross asynchrony of labelling with the coalesced nucleus always less heavily labelled than the chain of nodes (Fig. 4*d*). These results suggest that the gross asynchrony of DNA synthesis in graft complexes with cortical discontinuities is a consequence of the fact that coalesced nuclei synthesise less DNA than chain-form nuclei when sharing a common cytoplasm. The cortex therefore seems likely to regulate macronuclear DNA synthesis through some structural alteration associated with nuclear coalescence (such as a possible change in the structural state of chromatin and/or the macronuclear membrane).

Cortical control of nuclear asynchrony

It is clear from the experiments described here that identical nuclei sharing a common cytoplasm can be made grossly asynchronous with regard to morphology and DNA synthesis if they are associated with separate regions of cell surface. In *Stentor*, this association seems to involve physical attachment of the nucleus to a specific region of cortex[10]. Tartar's work[10] has indicated that the cell surfaces of regenerating and morphostatic *Stentor* differ in ways which are important for control of morphogenesis. My work provides further evidence for such differences and suggests their importance for control of nuclear morphology and synthesis. The finding that nuclear differences can be determined by cell surface differences is interesting in view of accumulating evidence that the ciliate cortex is regionally differentiated. In *Stentor*, the regions of wide and narrow cortical striping have different morphogenetic properties[10]; in *Paramecium*, cilia isolated from one mating type only adhere to cells of opposite mating type at specific cortical sites[15]. Most of these differences provide no clue as to how the cortex may control the behaviour of the nucleus. But the recent finding that the anterior and posterior regions of *Paramecium* respond differently to mechanical stimulation in terms of permeability to ions[16] suggests at least one specific way in which differentiated regions of cell surface might control the behaviour of underlying nuclei. Finally, in ciliates, nuclear differentiation is only one of many phenomena involving highly localised asynchronous behaviour of apparently identical organelles sharing a common cytoplasm. In *Nassula*, for example, the pre-existing cytopharyngeal basket is resorbed during cell division while two new baskets are forming for the daughter cells[17]; during reorganisation in *Stentor*, the original gullet dedifferentiates as a new one develops nearby[10]. My findings may be relevant to some of these phenomena as well as to nuclear differentiation in other cell types.

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The vegetation of Tertiary islands on the Ninetyeast Ridge

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Pollen from deep-sea drilling sites on the Ninetyeast Ridge which reflects island floras that flourished in the Palaeocene and Oligocene, shows pronounced similarity to Australian and New Zealand early Tertiary floras. Although they were closer to southern landmasses during the Tertiary, these islands were truly oceanic, and their colonisation occurred through long-distance dispersal mechanisms.

DEEP-SEA drilling on the Ninetyeast Ridge has shown the presence of shallow-water sediments at several points along the ridge crest[1,2]. At Deep Sea Drilling Project (DSDP) sites 214 and 254, in the northern and southern sectors of the ridge respectively (Fig. 1), sequences of volcaniclastics, sands, clays, pebble conglomerates, and organic-rich intervals lie immediately above the oceanic basement and show evidence of subaerial weathering and deposition. Among the strongest evidence for subaerial conditions is the presence of well preserved spores and pollen, reflecting the existence of a land vegetation of some diversity at points

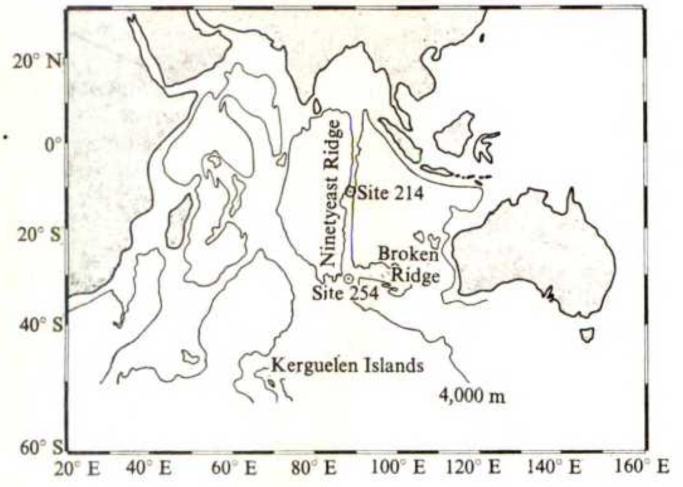

Fig. 1 Sketch map of the Indian Ocean showing the Ninetyeast Ridge and related features, and the position of Deep Sea Drilling Project sites 214 and 254.

along the ridge crest during the Palaeogene. We interpret such vegetation as that of oceanic islands that were emergent along the ridge from perhaps the latest Cretaceous to the late Oligocene; present fossil evidence establishes the presence of such vegetated land areas during the Palaeocene and Oligocene.

Preliminary accounts of the microfloras have already been published[3,4]; this paper documents the occurrence of these pollen-rich sediments, discusses the floristic composition of the vegetation they reflect, the dispersal problems faced by the island floras, and the influence on them of former distributions of land and sea areas. The detailed taxonomical classification of dispersed spores and pollen is currently being undertaken (Kemp and Harris, unpublished).

Tectonic setting of the Ninetyeast Ridge

The Ninetyeast Ridge extends for some 5,000 km along the 90°E meridian, from 32°S to at least 9°N. The ridge crest deepens progressively from south to north, and sediments immediately above oceanic basement at crest drilling sites increase in age northwards. The nature of sedimentary sequences at the drilling sites accord with a northward subsidence of the ridge throughout its history[5].

The ridge has been interpreted[6,7] as having formed from an extrusive pile accumulating at the junction of a former active spreading centre and a north–south trending transform fault, parallel to the present ridge. According to that model, volcanism was associated with motion along a transform fault between the Indian and Australian plates until their fusion in the late Oligocene. The formation of both the ridge and the attached Indian plate in relatively high southern latitudes and their subsequent northward movement is supported by magnetic anomaly data[8], by the palaeomagnetism of basalts at drilling sites[9], and by calcareous microfossils indicating warming of depositional conditions in younger strata[10].

Formerly emergent areas on the ridge crest are likely to have been relatively small islands, as the configuration of the ridge places limits on their east–west dimension, and the age differences of basal sediments from north to south precludes the emergence of large areas at any one time.

Stratigraphy of sampled sites

The generalised stratigraphy of sites 214 and 254 is shown in Fig. 2. At site 214, well preserved spores and pollen were recovered from interbedded volcaniclastics and lignites; this sequence is non-marine. These assemblages continue in to overlying glauconitic carbonate silts and sands which contain dinoflagellate cysts, acritarchs, foraminifera, nannofossils and molluscs. Foraminifera in the glauconitic unit indicate a Palaeocene age—P4 (ref. 11); the nannofossil zone of *Heliolithus kleinpelli* is present at 337 m below the sea floor, and the section may be as old as the *Cyclococcolithina robusta* zone at 389 m (ref. 12). This mid-Palaeocene date has been extrapolated down into the volcaniclastic sequence below as the spore and pollen assemblages are essentially the same throughout.

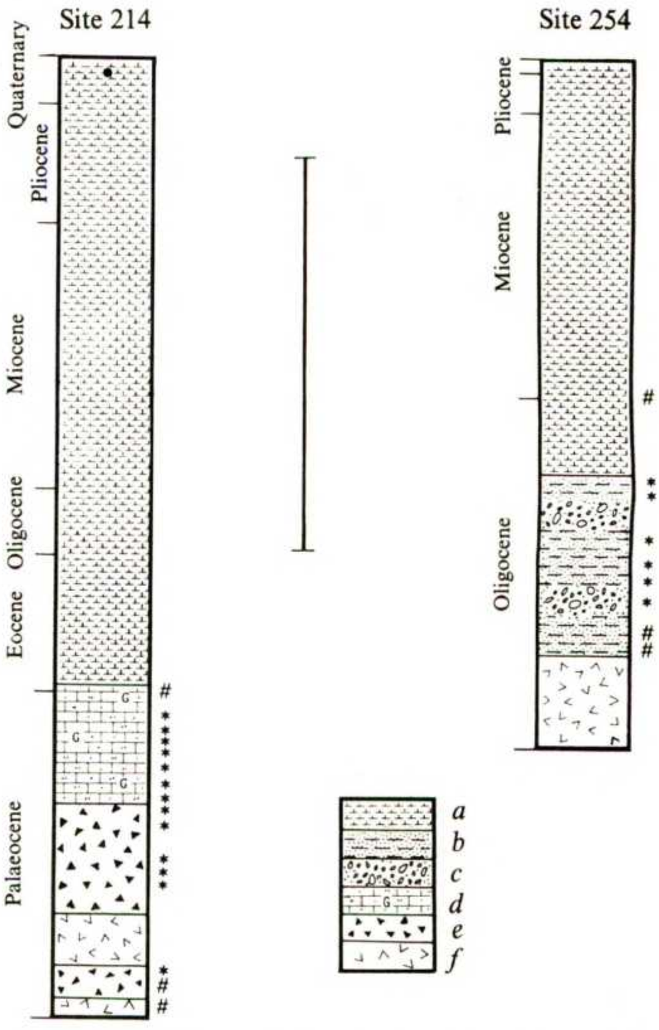

Fig. 2 Simplified stratigraphic columns at sites 214 and 254, with the position of spore and pollen-bearing core samples shown on the right of the columns. Modified from refs 10 and 12. Scale bar, 200 m; site 214, depth of penetration 500 m starting at a water depth of 1,665 m; site 254, depth of penetration 343.5 m starting at a water depth 1,253 m. *, Productive samples; †, barren samples; *a*, calcareous ooze; *b*, sandy and silty clay; *c*, pebble conglomerate; *d*, glauconitic carbonate, silt and sand; *e*, volcaniclastics interbedded with lignites; *f*, basalt.

At site 254, excellently preserved spores and pollen occur abundantly in the lowest sedimentary unit. This consists of poorly sorted sandstone and clay, some pebble conglomerates, has a high content of organic matter, and grades downwards into weathered olivine basalt. It possibly originates from the subaerial weathering of a basaltic terrain, with rapid deposition of weathering products in a quiet, shallow marine environment[10]; a lagoonal setting adjacent to vegetated highlands is perhaps represented. Age estimates of the palyniferous basal unit are ambiguous;

Table 1 Palynological form species of angiosperm and gymnosperm pollen recovered from sites 214 and 215, Ninetyeast Ridge, with their probable botanical affinity

Palynological form species	Probable botanical affinity	DSDP site 214	DSDP site 254
Angiosperms*			
Arecipites cf. *waitakiensis* (McIntyre)	Palmae		X†
Arecipites sp.	Palmae	X	
Australopollis obscurus (Harris)	Unknown	X	
Clavatipollenites hughesii Couper	Chloranthaceae (*Ascarina*?)	X	X
Cupanieidites orthoteichus Cookson and Pike	Sapindaceae (Cupanieae)		X
Echiperiporites sp.	Unknown		X
Gothanipollis cf. *gothani* Krutzsch	Loranthaceae		X
Halorgacidites cf. *harrisii* (Couper)	Casuarinaceae (*Casuarina*)	X	X
Milfordia homeopunctata (McIntyre)	Restionaceae (*Restio*?)		X
Myrtaceidites cf. *mesonesus* Cookson and Pike	Myrtaceae		X
Myrtaceidites spp.	Myrtaceae	X	X
Proteacidites cf. *symphonemoides* Cookson	Proteaceae		X
Proteacidites sp.	Proteaceae	X	
Nothofagidites spp.	Fagaceae (*Nothofagus*)	X	X
Psilotricolporites sp.	Unknown		X
Psilodiporites cf, *redundantis* Guzman	Moraceae/Urticaceae		X
Polycolpites sp.	Unknown		X
Sapotaceoidaepollenites rotundus Harris	Sapotaceae		X
Sparganiaceaepollenites sp.	Sparganiaceae		X
Spinizonocolpites prominatus (McIntyre)	Palmae		X
Schizocolpus marlinensis Stover	Didymelaceae (*Didymeles*?)	X	X
Striatricolporites sp.	Unknown		X
Tricolpites reticulatus Cookson	Gunneraceae (*Gunnera*)	X	X
Tricolpites spp.	Unknown	X	X
Tricolporites spp.	Unknown	X	X
Tubulifloridites cf. *antipodica* Cookson	Compositae (Tubuliflorae)		X
Tubulifloridites sp.	Compositae?	X	
Gymnosperms*			
Araucariacites australis Cookson	Araucariaceae (*Araucaria*?)		X
Lygistepollenites florinii (Cookson and Pike)	Podocarpaceae (cf. *Dacrydium*)	X	
Microcachryidites antarcticus Cookson	Podocarpaceae (cf. *Microachrys*)	X	X
Podocarpidites spp.	Podocarpaceae	X	X

*Only previously published form species are listed; new species are assigned to form genera only.
†X denotes presence.

the firmest control comes from the lower part of the overlying calcareous ooze unit, which has yielded middle to late Oligocene planktonic foraminifera[10]. Below that, foraminifera, ostracods and molluscs are not stratigraphically diagnostic, and an age range of Eocene to late Oligocene has been suggested[10].

Composition of the microfloras

Form species of angiospermous and gymnospermous origin present at the two sites are listed in Table 1. At site 214, the major pollen groups include 15 form species of angiosperms, 18 pteridophytes, 4 conifers and 3 lycopods. Palm pollens are dominant, and pollen similar to that of the extant New Zealand and New Caledonian genus *Ascarina* is abundant. Pollen of *Gunnera* type is consistently present. Several species occur so rarely that a wind-blown origin cannot be discounted; included here are *Nothofagus* (*brassi* type), Proteaceae, Casuarinaceae and Didymelaceae grain types.

The flora represented at site 254 was more diverse, with, as minimum figures, some 26 angiosperms, 3 conifers, 17 ferns, 2 lycopods and a variety of fungi. Tricolporate pollens of indeterminate affinity are most abundant. Among pollen groups identifiable to family level, Myrtaceae is most common. Two types were identified; one is broadly similar to that found in *Lophomyrtus*, *Rhodomyrtus* and related taxa; the other resembles no known living form. Palm types are again abundant; generic affinities are obscure, but similarities to *Cocos*, *Jessenia* and *Paralinospadix* are evident. Moraceae/Urticaceae pollens are a prominent element, and Casuarinaceae grains are present in quantities which suggest they are indigenous rather than wind-blown. Proteaceae and Restionaceae are both represented by types known in the Australian Tertiary. Families of global distribution are represented by Compositae and Graminae, both rare, and by Loranthaceae and Sapotaceae.

Conifer pollen at both sites suggests a largely podocarpaceous parentage. At site 254, coniferalean pollen makes up some 20% of the pollen spectrum. Araucariaceae types are common at this site, and have been tentatively identified as *Araucaria*. The fern spore spectrum at both sites is similar, and is dominated by cyatheaceous types, but Polypodiaceae, Gleicheniaceae and Schizaeaceae have been identified. Lycopod spores are rare, but three form species have been isolated. Of the abundant fungal remains at site 254, only fructifications of Microthyriaceae could be firmly identified.

Phytogeographical considerations

The plant microfossil assemblages indicate that the vegetation of these islands had much in common with the early Tertiary vegetation of Australia, New Zealand, southernmost South America and Antarctica. Few species occurred in common with Tertiary floras of India and Indo–Malaysian archipelago. Of 41 form-species of spores isolated at site 214, 28 have been identified, or have closely similar counterparts, in the Australian Tertiary. At site 254, 28 of the known 48 form species occur in the Australian Tertiary, and 19 are known from the Tertiary of New Zealand. Resemblances occur within all plant groups. Among conifers, the mixture of podocarpaceous and araucarian elements is typical of the Southern Hemisphere early Tertiary. Among angiosperms, species lists indicate that many common forms existed between continent and island, but qualitative differences lie in the extreme rarity of *Nothofagus* pollen at the island sites, and in the poor representation of Proteaceae.

Reconstructions of the Indian Ocean for the mid-Palaeocene and late Oligocene (Fig. 3) indicate that distances of the Ninetyeast Ridge islands from the southern

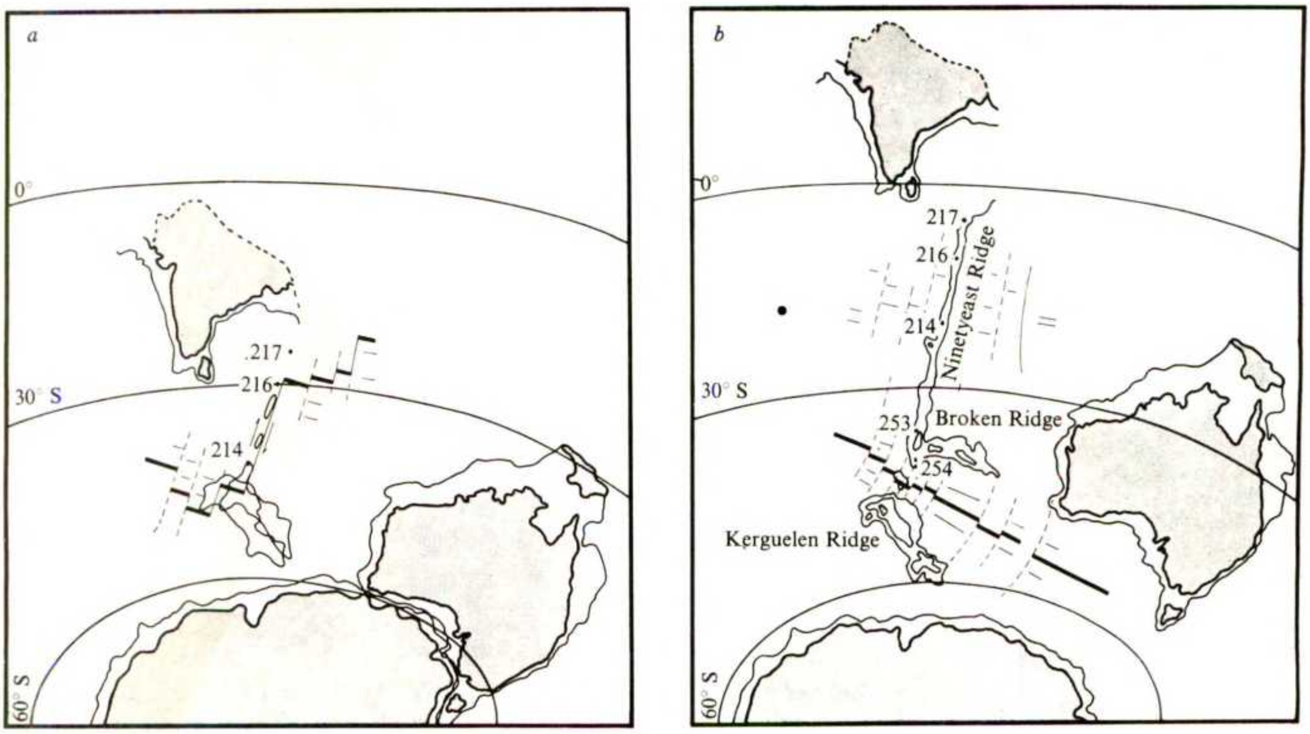

Fig. 3 Reconstructions of the eastern Indian Ocean for the mid-Palaeocene (*a*) and the late Oligocene (*b*), modified from ref. 7. Position of all Deep Sea Drilling Project drilling sites on the ridge crest is shown. Active centres of seafloor spreading are shown by heavy lines. Basalts at site 214 were being extruded approximately 60 Myr BP (*a*), along a transform parallel to the trend of the Ninetyeast Ridge; the Kerguelen and Broken Ridges are shown joined. At 25 Myr BP (*b*), when site 254 was emergent, a new axis of spreading, trending north-west separated Australia and Antarctica and Broken Ridge and Kerguelen. Site 214 has been spread to the north and submerged.

continents were much less in the early Tertiary in comparison with present geography, which must account in part for the similarity of the island floras with those of the landmasses to the south-east Islands which may have been emergent in the late Cretaceous would have been even closer to the continents. It does, however, seem likely that the sites were still truly oceanic, separated from the nearest continents by distances probably in excess of 1,000 km. The floras, therefore, must have become established through the long-distance dispersal of propagules, as has the vegetation of modern south temperate and subantarctic islands. Possible intermediate island sites, acting as biotic 'stepping-stones' between the southern continents and the ridge islands, may have been present along the formerly joined Broken and Kerguelen Ridges; beach gravels in a drilling site on Broken Ridge confirm that parts of it were emergent during the early Tertiary[2], and the Kerguelen islands have possibly been emergent since the Oligocene[13].

Problems in understanding the colonisation of the Ninetyeast Ridge islands relate to the apparent inability of some of the modern families represented to cross extended water gaps. Adaptations for long-distance wind and water transport are unknown in Casuarinaceae and Myrtaceae, and the present disjunct distributions of these and other southern families such as Proteaceae and Restionaceae are usually explained by invoking former land connections. *Araucaria*, too, has a modern distribution in the Pacific accountable in terms of previous land connections[14]. Migration pathways to the early Tertiary *Araucaria* stands in the Indian Ocean, on Kerguelen[15,16] and on the islands on the Ninetyeast Ridge, however, are more difficult to trace, and there are no Tertiary records from countries bordering that ocean. Podocarpaceae is another family poorly represented today on oceanic islands, although migration may have occurred through Indo–Malayan archipelagic islands during the late Tertiary.

Direct transport by winds and currents, and transport by biological agents are facilitated in regions of intense oceanic and atmospheric circulation, and in this context it should be noted that the islands of the Ninetyeast Ridge, when emergent, lay in the belt of circumpolar circulation. The intensity of this circulation must have varied throughout the Palaeogene; in the Palaeocene, with Australia and Antarctica still joined, and in the absence of a polar ice cap, circulation may have been relatively sluggish. By the Oligocene, however, in the wake of the opening of a seaway between Australia and Antarctica, and the development of an Antarctic icecap[17], strong westerly winds and currents probably prevailed.

Bird transport may have been significant in the establishment of many taxa on the Ninetyeast Ridge and Kerguelen sites. Birds must account for much seed transport to south temperate and subantarctic islands, although there have been few direct observations. Among the Ninetyeast Ridge microfloras there are some groups in which bird dispersal of seed is known—*Gunnera*, for instance[18], and members of the Loranthaceae are known to be dispersed by birds, and transport of *Podocarpus* fruits by frugivorous birds has been suggested[19], although the efficiency of such a dispersal mechanism over long ocean distances remains speculative. Moraceae, too, is a group with a high potential for this kind of dispersal and a possible example of the establishment of Myrtaceae (*Metrosideros*) on New Zealand shelf islands by migrating birds has been documented[20].

Comparison with modern island floras

The reconstructions of Fig. 3 suggest that the islands of the Ninetyeast Ridge, when emergent, lay somewhat south of 40°S. At present, in the eastern Indian Ocean, Amsterdam Island and St Paul Island lie at 37°55′S and 38°44′S, and Kerguelen Island lies at 49°30′S. The floras of these modern islands have few species; St Paul Island and Amsterdam Island at present support some 18 species of flowering plants[21,22], a figure somewhat lower than the 26 reflected in the pollen spectrum for site 254, which probably lay even farther south. Kerguelen Island supports about

21 flowering plant species. Modern island floras are further distinguished by a high ratio of pteridophytes to angiosperms—the occurrence of 'fern bush' communities on the more temperate of the southern oceanic islands, including Amsterdam Island, is a common feature[22]. On spore evidence, the pteridophyte assembly of the fossil islands seems comparable with those of the modern ones.

Even more striking than the apparently high diversity shown by the Tertiary island floras are the basic differences in floristic composition between them and the modern islands. Plants from high southern latitudes have been divided into "insular" and "continental" groups[23]; in the former category are taxa with a widespread distribution on southern oceanic islands; the latter include groups at present largely confined to the continents—the families Centrolepidaceae, Proteaceae, Restionaceae, Myrtaceae, and the genus *Nothofagus* have been cited as being chiefly of that distribution. Those groups are absent from modern islands in the southern Indian Ocean in spite of the existence of apparently suitable habitats on some of them. Such an absence has been taken by biogeographers to indicate a lack of dispersal ability.

The fossil evidence to hand suggests that members of some of these 'continental' families did in fact become successfully established on islands on the Ninetyeast Ridge, where pollen of Myrtaceae, Restionaceae, Proteaceae, and that of Podocarpaceae and Araucariaceae occurs in quantities that are not suggestive of a wind-blown origin. Geological considerations dictate that establishment at these sites must have involved dispersal across significant ocean gaps, so that these groups may not always have lacked vagility. It further suggests that their current absence from southern oceanic islands reflects the recent environmental history of those islands, all of which have been subjected, to a greater or lesser degree, to late Neogene chilling.

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letters to nature

Improved declination for transient X-ray source A0620−00

OBSERVATIONS with the Effelsberg 100-m telescope at a frequency of 4,600 MHz have resulted in an improved declination for the radio source believed to be associated with the transient X-ray source A0620−00 (ref. 1). The observations were made on August 24, 1975 with a cooled parametric amplifier of system temperature 65 K. The bandwidth was 200 MHz, and the half-power beamwidth of the telescope was 2.7′. The measurements were calibrated relative to NGC7027 and 3C286.

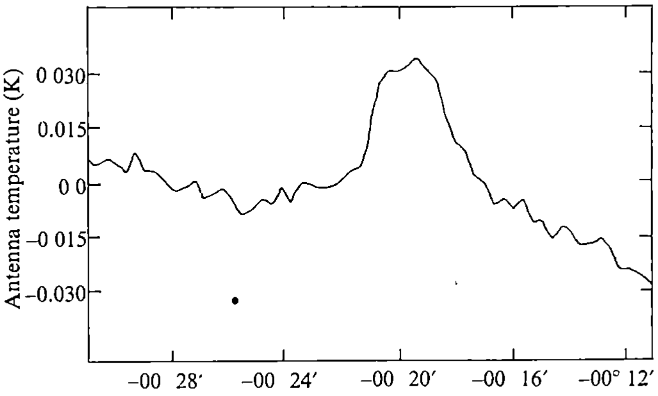
Twenty-one declination scans were made at right ascension (1950) 06 h 20 min 11.4 s, which was the interferometric position reported by Davis *et al.*[2]. Each scan was 20′ long, centred at declination (1950) −00°21′. The average of these scans is shown in Fig. 1. The baseline is not flat because of receiver instabilities (the system was operated without a Dicke switch), but the radio source is clearly present. The measured half-power width of 2.7′ is that expected for a point source. The declination (1950) of the source is −00°19′36±25″. The peak antenna temperature was 0.037±0.008 K, which corresponds to a flux density of 28±6 mJy at 4,600 MHz.

The declination measured here is consistent with the radio position reported by Craft[2], the optical position of Boley and Wolfson[3], and the X-ray positions given by Matilsky[3] and by Eyles *et al.*[2]. Our value for the flux density at 4,600 MHz is slightly larger than the 5,000-MHz value reported by Scott[4] for an observation two days earlier than ours, although the numbers agree within the uncertainties. Davies[5] reports that the flux density at 962 MHz was 21±9 mJy at the same epoch as our observation. The corresponding spectral index between 962 and 4,600 MHz is therefore

$$\alpha = 0.18^{+0.49}_{-0.38}$$

where $S(\nu) \propto \nu^{\alpha}$. This value suggests that the spectrum at cm wavelengths is relatively flat, in agreement with the flux densities measured by Owen *et al.*[4] at 1,418 and 2,695 MHz.

Fig. 1 Average of 21 declination scans made at RA (1950) = 06 h 20 min 11.4 s at 0850 UT, August 24, 1975. The half-power beamwidth of the telescope is 2.7′ at the observing frequency of 4.6 GHz. The peak source antenna temperature of 0.037±0.008 K corresponds to a flux density of 28±6 mJy for a point source.



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We scanned this position again on both September 7 and 8, 1975 but failed to detect the radio source. The data were of poor quality, but at that time we estimate that the flux density at 4,600 MHz was less than 20 mJy.

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X-ray sources and planetary nebulae

Current models of X-ray sources in binary systems generally assume accretion on to a neutron star, a black hole or a white dwarf as the mechanism of X-ray production. The accretion takes place from either a stellar wind or the inner Lagrangian point[1–5]. Here we explore the possible existence of a compact X-ray source in a binary system, where the companion star ejects its envelope to become a planetary nebula.

Consider first the possibility that the compact object is a neutron star. The accretion rate from a wind is given approximately by[6,7]:

$$\dot{M}_{\rm acc} = 1.8\times10^{19}\left(\frac{M_{\rm x}}{M_\odot}\right)^2\left(\frac{\dot{M}_{\rm pn}}{10^{21}\,{\rm g\,s^{-1}}}\right)\left(\frac{a}{10^{15}\,{\rm cm}}\right)\times$$
$$\times\left(\frac{V_{\rm p}}{10^6\,{\rm cm\,s^{-1}}}\right)\left(\frac{\bar{V}}{10^6\,{\rm cm\,s^{-1}}}\right)^{-3}{\rm g\,s^{-1}} \qquad (1)$$

where $M_{\rm x}$ is the mass of the accreting star, $\dot{M}_{\rm pn}$ the rate of mass ejection, a the separation between the stars, V the velocity of the wind and $\bar{V}=(V_{\rm r}^2+V_{\rm sound}^2)^{1/2}$ where $V_{\rm r}$ is the relative velocity between the accreting star and the wind. Assuming typical values for the mass of the ejecting star $\sim1.3M_\odot$, $a\sim10^{15}$ cm, $\dot{M}_{\rm pn}\sim10^{21}$ g s^{-1}, $V_{\rm p}\sim3\times10^6$ cm s^{-1}, we find: $\dot{M}_{\rm acc}\sim8\times10^{16}$ g s^{-1} which is in the range of typical ($\sim10^{17}$ g s^{-1}) accretion rates for compact X-ray sources. Thus, the X-ray luminosity of the proposed source is comparable to known X-ray source luminosities. The lifetime of such a source will be of the order of the ejection time $\sim10^2$–10^4 yr, unless the planetary phenomenon is a recurrent one. (Observations of FG Sag (ref. 8) imply that the planetary phenomenon may be a recurrent one.)

The question arises, however, whether a binary system in which one component is a neutron star and the other ejects a planetary nebula is a possible consequence of stellar evolution. We shall distinguish here a few possibilities.

For massive close binaries (A) stellar evolution estimates show[9] that a neutron star can be formed via a supernova explosion if the mass of the primary is greater than $16M_\odot$. This high mass is dictated by severe mass transfer during evolution. Stars with lower mass probably evolve into white dwarfs.

Consider such a system with masses of $16M_\odot$ and $2M_\odot$. The mass transfer process, which eventually occurs in such systems, lasts a few thousand years and yields a final primary mass of the order of[10]:

$$\frac{M_{\rm f}}{M_\odot}\sim10^{-0.96}\left(\frac{M_{\rm i}}{M_\odot}\right)^{1.4} \qquad (2)$$

For $M_{\rm i}\sim16M_\odot$, $M_{\rm f}\sim5.3M_\odot$ (we assume a loss of $\sim1M_\odot$ during the transfer process). A helium star with $5.3M_\odot$ evolves in $\sim10^6$ yr, and ends its evolution as a supernova. This time is too short for any appreciable evolution of the secondary star.

Present theories of planetary formation do not consider stars as massive as our companion star ($\sim11.7M_\odot$) as possible progenitors of planetary nebulae (however, since these stars are so rare compared with stars of $\sim1.4M_\odot$ it is impossible to exclude the possibility of their becoming planetary nebulae on observational grounds). One would, therefore, like to see whether it is possible for the supernova ejecta to blow off most of the mass of the second star. The answer depends on the energy required and the efficiency of the process.

The binding energy of the outer layer is:

$$E_{\rm b}\sim3.8\times10^{48}\left(\frac{M}{M_\odot}\right)^2\left(\frac{R}{R_\odot}\right)^{-1}{\rm erg} \qquad (3)$$

where M is the mass of the outer layer and R the radius of the star.

The energy needed to separate the two stars to the distance required by the correct accretion rate is approximately:

$$E_{\rm s}\sim9.1\times10^{47}\left(\frac{M_1'}{M_\odot}\right)\left(\frac{M_2'}{M_\odot}\right)P^{-2/3}\left(\frac{M_1+M_2}{M_\odot}\right)^{-1/3}{\rm erg} \qquad (4)$$

where P is the period (in days) before separation; M_1, M_2 the masses before explosion; M_1', M_2' the masses after explosion. On the other hand, the available kinetic energy going into the cone spanned by the second star is:

$$E_{\rm a}\sim1.4\times10^{49}\left(\frac{M_{\rm ej}}{M_\odot}\right)\left(\frac{V_{\rm ej}}{10^9\,{\rm cm\,s^{-1}}}\right)^2\left(\frac{R_2}{R_\odot}\right)^2\times$$
$$\times\left(\frac{M_1+M_2}{M_\odot}\right)^{-2/3}\left(\frac{P}{1\,{\rm d}}\right)^{-4/3}{\rm erg} \qquad (5)$$

Putting in some typical values: $M\sim10M_\odot$, $R\sim5R_\odot$, one obtains $E_{\rm b}\sim7.6\times10^{49}$ erg (this should be reduced since the outer envelope is not so tightly bound). For $M_{\rm r}+M_1\sim17M_\odot$, $M_1'\sim M_\odot$, $M_2'\sim1.3M_\odot$, $P\sim1$ d, we find $E_{\rm s}\sim4.6\times10^{47}$ erg. Taking: $M_{\rm ej}\sim4M_\odot$, $V_{\rm ej}\sim0.5\times10^2$ cm s^{-1}, $R_2\sim5R_\odot$, $M_1+M_2\sim17M_\odot$ and $P\sim1$ d, we get: $E_{\rm a}\sim5.3\times10^{49}$ erg. This seems energetically possible, but detailed calculations show that the efficiency of this process is very low (E.E. Salpeter, personal communication). Consequently, if we start with a close binary and neutron stars are formed only by supernova explosions, and only relatively low mass stars are capable of ejecting a planetary nebula, then the probability to form such an X-ray source is small.

For low mass widely separated binaries ($a\sim10^{15}$ cm, called case B) a different type of evolution is obtained. In this case the minimum mass for producing a neutron star is $\sim4M_\odot$ whereas the secondary can have initially $M\sim1.2M_\odot$.

Another possibility, to be called case C, is to assume the accreting compact X-ray source to be a white dwarf[11,12]. (The problem of the production of the X rays can be overcome since an X-ray tail is obtained from an accreting white dwarf, similar to the γ-ray tail found in neutron stars (S. L. Shapiro and E. E. Salpeter, unpublished)). Clearly no supernova phenomenon is called for here. The accretion rate required to provide the observed luminosities (10^{36}–10^{37} erg s^{-1}) demands a separation of at most 5×10^{13} cm. The emission, however, will be mostly in the ultraviolet with some X-ray emission.

Assuming that X-ray sources of the types described, exist in our Galaxy, we evaluate the probability of observation for each case.

For A, a neutron star can be formed if $M_{\rm primary}\gtrsim16M_\odot$. The estimate of the number of such binaries in the Galaxy (after mass transfer) is about 4,000 (ref. 9). The He star evolves in

Table 1 Possible coincidences between X-ray sources and planetary nebulae

Source	Name	Right ascension (h min s)	Declination (° ′ ″)	Area of X-ray error box (degree^2)	Comments
X-ray	3U1735–28	17 35 24	−28 27 0	0 04	Known to be a transient source
Planetary nebula	Th 3–35	17 35 5	−28 41		
X-ray	3U1735–44	17 35 12	−44 25 12	0.002	—
Planetary nebula	Fg 2	17 35 7	−44 08		
X-ray	3U1714–39	17 14 55	−39 18 00	0.083	Nebula probably old
Planetary nebula	H2–6	17 14 56	−39 16 10		
X-ray	3U1746–37	17 46 48	−37 00 36	0.0184	Counterpart globular cluster NGC 6441?
Planetary nebula	H1–36	17 46 24	−37 00 36		

$\sim 10^6$ yr whereas the secondary ($\sim 1.4 M_\odot$) requires $\sim 10^9$ yr before ejecting its envelope. The ejection itself (and therefore the X-ray stage) lasts at most $\sim 10^4$ yr. Thus the probability of discovery is negligibly small.

In case B with $M_{\rm primary} \gtrsim 4M_\odot$, $M_{\rm secondary} \sim 1.2$–$1.4 M_\odot$, there are $\lesssim 7\times10^4$ such binaries in our Galaxy[13]. Taking into account the relative lifetime of the X-ray phase compared to stellar evolution, we expect the number of such sources in the galaxy to be about one.

Finally in case C, we assume the X-ray source to be a white dwarf. The expected number[13] of such sources is ~ 70, (these numbers should of course be multiplied by the ratio of the accessible volume in X rays and visible to the total galactic volume).

Dust formation during the early stage of envelope expansion gives rise to infrared radiation. It is expected, therefore, that X-ray sources powered by accretion from a young planetary nubula should be infrared sources.

An interesting X-ray source which possibly coincides with an infrared and radio source is[14,15] Cyg X-3. The small separation ($P = 4.8$ h) requires a low mass ejection rate to avoid total extinction of the source. For $\dot{M}_{\rm pn} \sim 10^{19}\ {\rm g\,s^{-1}}$ we obtain $\dot{M}_{\rm acc} \sim 3.8\times10^{20}\ {\rm g\,s^{-1}}$, which is too high, but regulation by the Eddington limit may reduce this value to the required one.

A search for possible coincidences between observed X-ray sources[17] and observed planetary nebulae[16] yielded a few candidates, the best of which are given in Table 1 with some comments.

To conclude, the probability of observing an X-ray source in the form of a compact object accreting from an expanding planetary nebula is sensitive to the nature of the compact object (black holes have not been discussed) and to the course of evolution. We expect in such systems a coincidence between the X-ray source (or ultraviolet source) and infrared sources (or very young planetaries). The end product of a planetary nebula and a white dwarf should be a double white dwarf binary, one such system—LDS275AB—is known[18] (with 4″ separation and having $m_v = 15$, $M_v = 13$ corresponding roughly to a separation of $\sim 1.5\times10^{15}$ cm) and there are a few other possible candidates for such systems.

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Qualitative features of synchrotron radiation in an inhomogeneous magnetic field

A UNIFORM, large scale magnetic field is probably an abstraction not found in nature. All sorts of instabilities may arise, and a filamentary or sheet-like structure is most likely to ensue. For example, it is well known that a picture of the optical continuum of the Crab Nebula shows a cotton-like structure, on a scale much smaller than the wisps ($\sim 10^{16}$ cm).

If the magnetic field is non-uniform, the estimates of the lifetime of energetic electrons against synchrotron losses must be modified, whereas for the low energy radiation there occurs another phenomenon. This is most easily seen if we suppose that the magnetic field is confined to plane sheets of average thickness R and total volume V_0. The magnetic field, H, is supposed to be constant in the sheets, and zero outside.

The total power radiated by the system of sheets is $L(\nu) = NV_{\rm eff}(\nu)P(\nu)$, where N is the number of sheets, $V_{\rm eff}(\nu)$ is the effective volume per sheet radiating at a given frequency, and $P(\nu)$ is the power at given frequency emitted per cm^3. The point is that the effective volume depends on the energy of the electrons (which are supposed to be injected from the outside) and therefore on the frequency. In fact low energy electrons can penetrate into the sheets only up to a distance of the order of the cyclotron radius r_c.

The effective volume is therefore $V_{\rm eff} = V_0 r_c/R$. Now, $r_c \propto \gamma \propto \nu^{1/2}$, where γ is the Lorentz factor. Therefore

$$L(\nu) \propto NV_0\nu^{1/2}P(\nu) \qquad (1)$$

The usual approximation has been used in which the synchrotron spectrum per electron is a delta function.

From the energy at which $V_0 = V_{\rm eff}$ onwards, the spectrum is $P(\nu)$. This energy corresponds to $r_c = R$, and sets the position of the low energy break.

If the magnetic clouds have different shapes, there may be a smoother break. For example, it is easy to show that spherical cloudlets offer an effective volume

$$V_{\mathrm{eff}}(r_c) = V_0[3(r_c/R) - 3(r_c/R)^2 + (r_c/R)^3] \qquad (2)$$

Again, at low energies, the term r_c/R dominates.

Summing up, this mechanism flattens the low energy spectrum by a half-power law; the specific shape of the break depends on the geometry of the magnetic inhomogeneities, and on the dispersion of their sizes. The fact that the radiated power per electron must in this case be multiplied by a factor γ has been noticed by Tucker[1], but not exploited, as far as we know. From the low energy break onwards, the spectrum follows its theoretical $P(\nu)$. A second break then ensues when the energy losses of electrons by radiation become important. It is well known that this happens for a Lorentz factor $\gamma = 5.2\times10^8 H^{-2} T_{\mathrm{eff}}^{-1}$, in Gaussian units. In this case, of course, T_{eff} is not equal to T, the lifetime of the nebula, but is the fraction of T which the electrons spend in the magnetic clouds. For high energy electrons, and in the case of spherical cloudlets, $T_{\mathrm{eff}} \sim 2TR/\Lambda$. In this expression Λ is defined in such a way that $\Lambda - 2R$ is the customary mean free path. When $2R = \Lambda$, the field is uniform, and setting $2R > \Lambda$, of course, does not make any sense.

As an application of what we said above, we will show that it is easy to find a reasonable set of parameters H, R, Λ which can reproduce the gross features of the spectrum of the Crab Nebula. For gross features we mean first, the radio spectrum, which extends up to about $\nu = 3\times10^{13}$ Hz, with power law index not too different from 0.3; second, the optical spectrum, with a power law index compatible with the value 0.8; third, the high energy spectrum, from 3.2×10^{14} Hz on, where a power law index of 1.3 can well represent the data[2].

We then have the following equations

$$\begin{aligned}
\nu_1 &= 1.2\times10^6 H_\perp \gamma_1^2 = 3\times10^{13}\\
\nu_2 &= 1.2\times10^6 H_\perp \gamma_2^2 = 3.2\times10^{14}\\
\gamma_1 &= 7\times10^{-4} H_\perp R\\
\gamma_2 &= 0.009(\Lambda/R) H_\perp^{-2} \qquad (3)
\end{aligned}$$

As usual, $H_\perp$ indicates the component of the magnetic field normal to the electron trajectory. We have assumed that the magnetic fields are randomly oriented, and therefore $H_\perp = \sqrt{(2/3)}H$. Setting the magnetic field $H = h_0\times10^{-3}$ gauss, gives the following:

$$\begin{aligned}
R &= 2.44\times10^{11} h_0^{-3/2}\ \mathrm{cm}\\
\Lambda &= 1.03\times10^{13}\ \mathrm{cm}\\
\Lambda/R &= 42.4 h_0^{3/2} \qquad (4)
\end{aligned}$$

From the last expression we see that $h_0 \geqslant (21.2)^{-2/3} = 0.13$ for the model to make sense.

To verify that the energetic requirements of the model are tolerable we estimated E_e, the total energy of the electrons, E_h, the total energy of the magnetic field, and $\delta \doteq E_h/E_e$, which should tell how far we are from equipartition. We assumed, for simplicity, a uniform distribution of spheroidal cloudlets. In this case, the total volume of the clouds is given by $V_0(R/\Lambda)$, since the average distance between clouds is approximately given by $(R^2\Lambda)^{1/3}$. The distance we assumed is 2 kpc, and V_0, the total volume of the (radio) nebula, is 3×10^{55} cm^3, according to the data given by Apparao[3]. With these assumptions we get: $E_e \sim 2.6\times10^{50}$ erg; $E_h \sim 2.8\times10^{46} h_0^{0.5}$ erg and $\delta \sim 1.1\times10^{-4} h_0^{0.5}$. For different shapes and dispositions of the clouds (filaments or sheets) these parameters would depend on different powers of h_0, and we feel that the situation could well move closer to equipartition.

The foregoing model is not as limited in scope as might appear at first sight. We will now show that a system of filaments with a more general magnetic field distribution, must give, to a first approximation, a similar flattening of the spectrum. Consider the radiation from a single filament, where the magnetic field as a function of the axial distance r is given by the law $H \propto r^{-\alpha}$. There are two restrictions: α must ensure a sufficiently fast decrease of the magnetic field, and because an infinite magnetic field is nowhere acceptable, we must expect that the field is truncated at $r = R_0$. The character of the spectrum is most easily seen if we suppose that $H = 0$ for $r < R_0$. We then make rather crude approximations, with the result that the total power radiated is given by the law

$$L(\nu) \propto \int_{r_{\mathrm{min}}}^{\infty} \gamma_\nu^2 H_\perp^2 \gamma_\nu^{-m} \gamma_\nu \nu^{-1} r\,\mathrm{d}r \qquad (5)$$

Here, $r_{\mathrm{min}} = \max(R_1, R_0)$, where $R_1 \propto \nu^{1/(2-3\alpha)}$ and m is the index of the electron distribution, which we take as 2.6. The expression for R_1, the counterpart of the geomagnetic cutoff, is easily found by setting the cyclotron radius equal to the axial distance r, and keeping in mind that

$$\nu \propto H_\perp \gamma^2 \propto H_\perp (Hr)^2 \propto r^{-\alpha}(r^{1-\alpha})^2$$

It then seems that for $R_1 < R_0$, or $\nu > aR_0^{(2-3\alpha)}$, where a is a suitable constant, the spectrum follows the customary power law $\nu^{-(m-1)/2}$. On the contrary, for $\nu < aR_0^{(2-3\alpha)}$, there appears an extra factor ν^β, where

$$\beta = \frac{2-\alpha[(m+1)/2]}{(2-3\alpha)}$$

The numerical value of β depends both on α and on m. For $m = 2.6$, it is easy to see that β has the asymptotic value of 0.6, and for $\alpha = 2$ it takes the value 0.4.

We have here merely tried to show that it is possible to achieve a reasonable fit for the spectrum of the Crab Nebula, without worrying, for the present, about other features, and without attaching too much weight to the values obtained for R and Λ. We thus lose equipartition, but we find a simple explanation for two breaks, and, perhaps more important, we show that an injection spectrum of electrons with power law index 2.6 is acceptable.

It is interesting to note that we are left with one free parameter, namely h_0. With this parameter in hand, we can adjust the magnetic field so that it is not in conflict with the γ-ray observations of the Crab Nebula[4]. We have numerically estimated the Compton–synchrotron radiation by means of the complete formulae found in the review paper by Blumenthal and Gould[5]. A value of $H \sim 10^{-2}$ gauss is largely sufficient to avoid an excess of high energy γ rays, whereas $H = 10^{-3}$ gauss is on the border line.

Whether the foregoing model is really valid for the Crab or not, we think that the situation described here may occur in many synchrotron emitting sources.

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Thorium in the spectrum of HR465

THE peculiar A star HR465 is a spectrum variable[1]. Considerable attention[2,3] has been given to the spectra of this star obtained by W. P. Bidelman during 1960–61, when the lines of the rare earth elements attained their maximum intensity; Bidelman has kindly placed this material at our disposal. Among the remarkable aspects of the HR465 spectrum is the fact that it is the only star for which a firm statistical basis exists for the identification of uranium[4].

Bidelman (unpublished) has assigned a moderate spectral feature in HR465 at λ4019.12 to Th II λ4019.13, which is the strongest accessible thorium line. The occurrence of this line in stellar spectra is very unusual. We base this statement on an extensive series of wavelength measurements made by C.R.C. and G.C.L.A. on 2.4 Å mm^{-1} plates taken at the Dominion Astrophysical Observatory. Combining these results with previous work[2,5], we have measured wavelengths in some 50 Ap and Am spectra, many of them very rich and sharp-lined, and only HR465 shows a line within 0.04 Å of λ4019.13.

The thorium identification can be based on more than the single line λ4019. We have re-examined the statistics of wavelength coincidences using the Th II wavelengths and intensities given by Meggers, Corliss and Scribner[6]. Coincidences within ±0.04 of stellar wavelength with Th II are significant at the 95% confidence level or higher. The highest significance (99.5% confidence) was based on four coincidences within ±0.02 Å using a list of seven Th II lines with laboratory intensities greater than 1,000. These results are somewhat more favourable than those obtained earlier[2].

We have obtained an abundance for thorium in HR465 at rare-earth maximum from this line using the method of Cowley and Hartoog[4]. The gf values were taken from Corliss and Bozman[7] or computed from the data in Meggers *et al.*[6] using atomic data supplied by Corliss.

This method shows a remarkable consistency. For example, we determined the thorium–neodymium abundance ratio using λ4018.69 of Nd II, and the thorium–gadolinium ratio using λ4013.80 of Gd II. When these ratios were converted to absolute abundances using Aller's[1] analysis of HR465, the results are within 0.2 in the logarithm. Other abundance determinations[8] show a similar self consistency.

The absolute abundance that we derive for Th is 4.4 ($\log H=12.0$). This should be compared with the uranium abundance of 4.6, which we now revise from ref. 4 on the basis of a new second ionisation energy for uranium[9]. We believe this result to be correct to slightly better than an order of magnitude.

If we assume that the present Th–U ratio of −0.2 dex (±roughly 0.7 dex) in HR465 is the result of radioactive decay following the nucleosynthetic event, then we can say that the event must have taken place more recently than the time of the birth of the Galaxy, some 10^{10} or more years ago.

Certain non-nuclear[10,11] theories of the origin of abundance anomalies in Ap stars would obfuscate any conclusion of this kind. But these theories have not been developed to the point where they can make definite prediction of the relative abundances of heavy nuclides. On the other hand, it has been shown[8] that the relative abundance pattern of the heavy nuclides in HR465 resembles the prediction of the r-process. We shall, therefore, proceed with a nuclear interpretation, which at present provides the most complete and detailed basis for an understanding of the observations.

Cameron[12] gives 0.67 for the logarithm of the solar system thorium–uranium ratio. Our result differs from this by 0.87 in the logarithm. If these figures could be taken at their face value, there would be a clear indication from the work of Blake and Schramm[13] that the HR465 material was processed less than 10^9 yr ago while the Solar System r-process ceased more than 10^9 yr ago. Unfortunately, the uncertainty of the present determination will not permit this interesting conclusion, and we must content ourselves with the statement that thorium and uranium were made in HR465 at some time more recently than the formation of the oldest globular clusters.

The significance of this crude cosmochronological result lies in the fact that it depends on observations of an object outside of the Solar System.

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Controlled artificial generation of geomagnetic pulsations

THERE has been a shift away from purely passive studies of the ionosphere and magnetosphere to more active studies in which the response of these regions to a controlled man-made disturbance is determined by experiment[1,2]. One area of research where active experiments may be particularly useful is in the study of ultra low frequency (u.l.f.) waves in the ionosphere and magnetosphere. We do not yet completely understand these waves from observations of the geomagnetic pulsations they produce because of the great difficulty in separating source and propagation effects. This problem is particularly acute for the u.l.f. waves generating Pc 1 geomagnetic pulsations (0 2–5 Hz) where the wavelengths are small compared with the dimensions of the magnetosphere. What is needed is a controlled

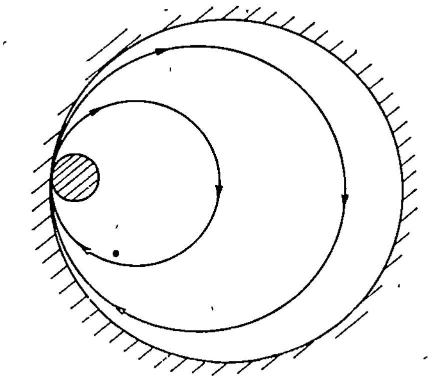

Fig. 1 Large scale representation of the island/circular sea model. The island is connected to the shore of the sea by a narrow insulating neck of length 100 m at the point of closest approach. Other relevant dimensions are: island radius 10 km, sea radius 100 km, sea depth 50 m, total current between electrodes on either side of the neck 3,000 A Two of the circular current flow lines in the sea are indicated by arrows

source of u.l.f. waves which would enable source and propagation effects to be separated. We believe we have found a technically feasible and comparatively inexpensive method for the controlled artificial generation of u.l.f. waves.

A number of suggestions have been made for such a generator, suggestions include a large ground-based current loop, or a large horizontal antenna[3–7]. Artificial stimulation of u.l.f. waves by direct modification of currents in the lower ionosphere has been proposed[8]. Another method uses very low frequency (v l f) radio transmissions into the magnetosphere to stimulate u.l.f. waves[9–11]. The ground-based current loop method would probably be one of the most reliable and versatile but, to generate observable pulsations, the area of the loop must be large and the power requirement is many MW. We here propose a new version of the ground-based current loop method, in which an electric current is passed around a peninsula in an enclosed sea or large saline lake. We describe model calculations which demonstrate that significant magnetic field variations can be generated in the lower ionosphere for powers of the order of 1 MW. It has been shown theoretically that this can produce u.l.f. waves in the ionosphere[3,7].

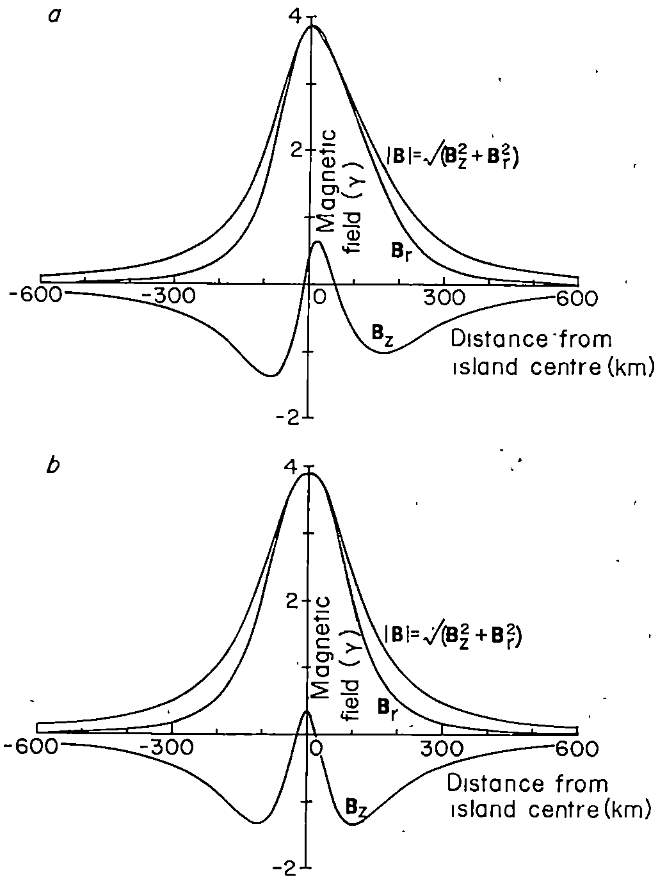

Fig. 2 The magnetic field generated at 100 km altitude. *a*, Distance from island centre measured perpendicular to the shore; *b*, distance from island centre measured parallel to the shore.

The model analysed consists of a small circular island connected to the shore of a large circular sea by a narrow neck (Fig. 1). All sea boundaries are assumed to be insulating. Physical dimensions are chosen as follows: island radius 10 km, sea radius 100 km, neck length 100 m, sea depth 50 m. An electric current flows around the island between electrodes which are, for simplicity, assumed to completely cover either side of the neck. The current distribution for a given potential difference is obtained by solving Laplace's equation using standard conformal mapping techniques. The flow lines are circular and the sea can be divided into circular current elements of infinitesimal width. The ionospheric magnetic field is obtained by integrating over the contributions of all elements. The sea is required to be bounded so that the inductance is finite. The inductance calculation is difficult, and an estimate was made from measurements on a scale model .

The relevant parameters are as follows: resistance 0.246 Ω, maximum current density 0.6 A m^{-2}, power at 1 Hz–2.39 MW, self inductance ≈20 mH. Magnetic fields generated in the ionospheric E region are shown in Fig. 2. At u.l.f. frequencies, simple lumped circuit analysis may be applied; at 1 Hz, the circuit has an impedance of 0.27 Ω and the power required to pass a current of 3,000 A through the seawater is 2.39 MW. The maximum current density (at the electrodes) is 0.6 A m^{-2}; over most of the sea the current density is much smaller: on the far side of the island it is only 0.01 A m^{-2}. The amplitude of the magnetic field generated is more than 2.5γ over 10,000 km^2 at an altitude of 100 km. This magnetic field amplitude is greater than that of most naturally occurring micropulsations.

There are several idealisations in our model. Perhaps the most important is the assumption that the sea boundaries are insulating. Clearly, the currents through the neck and adjacent sea floor must be minimised. Seawater conductivity is typically 4 S m^{-1} and for a location where the island, neck, and sea floor consists primarily of solid rock such as granite, with a conductivity of less than 10^{-2} S m^{-1}, approximately 50% of the power is lost by conduction through the neck. This could be reduced by insulation placed behind the electrodes. Alternatively, a freshwater lens beneath the neck and island would reduce the conductivity substantially. Furthermore, currents induced in the sea floor by the changing magnetic field generate an opposing magnetic field For a conductivity of 10^{-2} S m^{-1}, and frequency 1 Hz, the skin depth is 5.0 km, and for a current loop the maximum axial magnetic field produced at an altitude of 100 km will be reduced by a factor of 6 from the ideal value[5] obtained assuming zero conductivity. Even reduced by this factor the fields generated are still greater than most naturally occurring micropulsation amplitudes.

The other idealisations in the model are less likely to be important in practice. The shapes of the peninsula and sea are unimportant, as shown in model calculations, provided the area enclosed by the current flow lines is large. There exist a number of saline lakes and enclosed seas that would be suitable; for example, the Great Salt Lake in the USA. The use of electrodes which completely cover the neck is usually impractical. Smaller electrodes will produce an increased voltage gradient in the immediate vicinity of the electrodes, possibly necessitating fencing to exclude sea life. Farther from the electrodes deviations from the idealised case will be insignificant. We emphasise that the resulting voltage gradients are very small. For example, in our model a swimmer opposite the neck of the island would experience a voltage difference of 0 03 V from head to toe (a distance assumed to be 2 m).

An important distinction between this new version of the ground-based current loop method for generating u.l.f. waves and the original method is its use of the conducting properties of the 'ground' (that is, seawater), resulting in reduced construction and operation costs and more available locations. Once a suitable peninsula has been located, the insertion of electrodes, switching circuitry and transformers and the provision of a modest amount of power ($\sim$1 MW) should allow the generation of distant u.l.f. waves of observable magnitude.

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Reality and nature of a Sun–weather correlation

WILCOX *et al.*[1,2] have claimed that some influence associated with the solar magnetic sector structure produces an effect in the terrestrial troposphere, which is manifested by changes in the vorticity area index (VAI). (The VAI is a measure of the strength of the cyclonic circulation in the Northern Hemisphere.) The phenomenon was revealed by means of a superposed-epoch analysis of the VAI data which made use of 54 key days, determined by the solar magnetic data, and produced a mean variation about the key days (Fig. 1—our reconstruction of Fig. 5 of ref. 2). Wilcox *et al.* concluded that the effect lasts for a few days and introduces changes of up to 10% in the VAI, but occurs only in the winter months.

Reports of short term Sun–weather correlations have been greeted with scepticism by many. We have, however, subjected the VAI data (provided by W. O. Roberts; determined for the 3×10^4 Pa (300 mbar) level) of the original analysis to a variety of statistical tests, and we have requested and obtained a subdivision of new data that have been reported[3] in furtherance of the claim. (J. M. Wilcox and L. Svalgaard, personal communication. They extracted from the analysis in ref. 4 results both for the 81 new cases included there, and for the 46 whose key days could be clearly identified from spacecraft observations alone.) We find ourselves obliged, however, to accept the validity of the claim by Wilcox *et al.*, and to seek a physical explanation.

In the course of our statistical tests, we had determined that the alleged solar 'signal' was stronger during periods of relatively strong meteorological 'noise' (as measured by the amplitude of VAI variations, indicative of transient large scale weather systems); a 'signal' amounting to a 30% change of VAI was detected in the case of a superposed-epoch analysis of the 12 'noisiest' key days, for example. This had seemed to indicate that the alleged signal might be merely residual noise, inadequately cancelled in the superpositioning If the reality of the correlation is accepted, however, quite a different inference must be drawn. Either the meteorological noise is, in large part at least, the solar signal, or else the solar influence (whatever its nature may be) must somehow modulate the meteorological noise to produce its signal. The first option is generally discredited on grounds of simple energetics, and it is probably ruled out by observational evidence, even if 'trigger' mechanisms are invoked. The second option does not suffer from these difficulties, however, and we investigate it here.

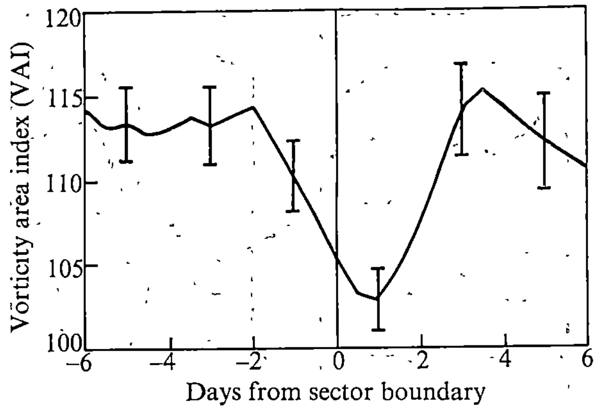

Fig. 1 Variation of vorticity area index (VAI, in units of 10^5 km^2, determined for the 3×10^4 Pa pressure level) with days measured from the passage of a solar magnetic sector boundary past the Earth: a superposed-epoch result incorporating VAI data associated with 54 sector boundaries, reconstructing the variation depicted by Wilcox *et al.*[2].

Phase modulation is the simplest kind we can propose and we have tested it by simulation methods. We suggest that the solar influence can selectively accelerate and/or decelerate the natural amplitude variations of VAI in such a way that minimum VAI tends to be found near to the times of the passage of solar magnetic sector boundaries past the Earth, rather than being randomly distributed. The physical mechanism by which such phase shifts might be introduced remains unknown.

To test this hypothesis by simulation, we have chosen at random 54 artificial key dates (and other numbers of artificial key days) in a variety of ways from among all the winter days in the period January 1, 1964 to December 31, 1970—the period used by Wilcox *et al.*[2] We then shifted the key days forwards or backwards by 12 or 24 h, to minimise the VAI that would have been obtained on the key day. Both the purely random and the adjusted sets of artificial key days were then used in superposed-epoch analyses of VAI. The phase adjustment was found to produce patterns having a well defined signal from the originally random data (Fig. 2). The upper set of traces depicts results for 54 key days; the lower set depicts results for 131 key days, the number now in use[3]. Between the two sets, on the right, is the original signal.

The simulation signal we have produced in this way constitutes a signature determined by, and characteristic of, the VAI data, in combination with the rules of phase adjustment alone. The solar influence, whatever its nature, produces a signature of its own that is clearly of much the same character. The signatures could be forgeries of one another; but, if they are not, the solar influence must be producing its signature very largely by a phase shifting of meteorological noise.

We are at present testing variants on the rules of simulated phase shifting in order to duplicate more successfully the solar-induced signature and to determine the range of rules that might apply. Hypotheses alternative to the phase-shift hypothesis, but consistent with our basic contention that meteorological noise is being modulated, are also under consideration. Our detailed results will be reported shortly.

The concept of a phase-shifting or similar modulating mechanism very largely avoids, *a priori*, difficulties normally associated with the energetics of a Sun–weather relationship: the usual sources and sinks of meteorological energy continue to be called on, and it is only the timing of the

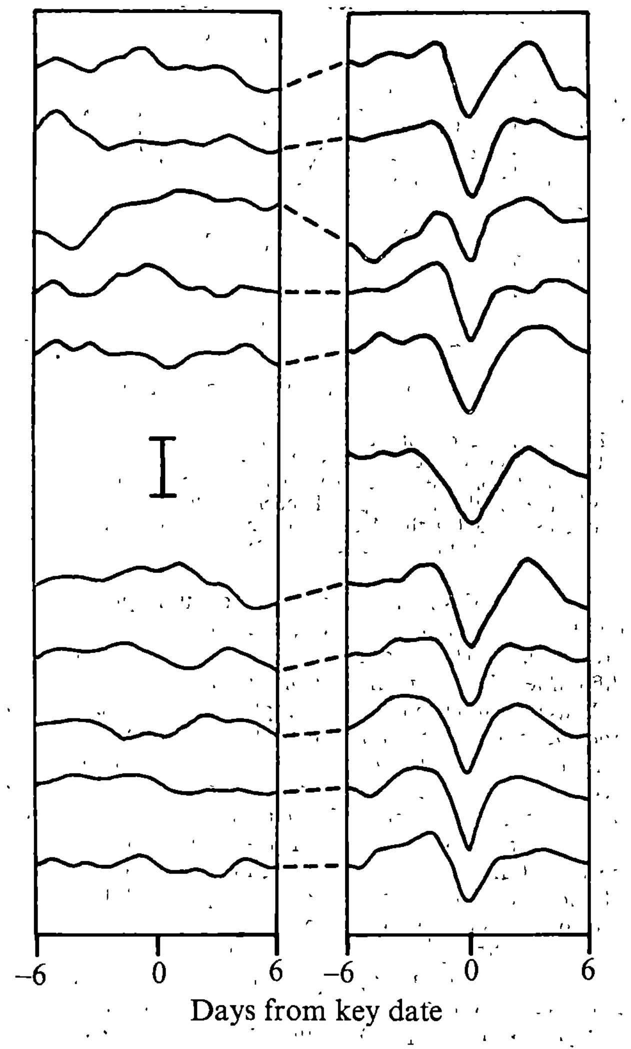

Fig. 2 Variation of VAI with days measured from key dates that are selected at random (left panel) and from key dates that are adjusted as described in the text from the randomly selected key dates (right panel). The upper five curves use 54 key dates and the lower five curves use 131 key dates. The vertical bar in the left panel represents 10 units (of 10^5 km^2) in the VAI. The curve opposite it in the right panel is the curve of Fig. 1, replotted on the present scale and shifted one day to the left (to match the criteria imposed for the adjustment of the randomly selected days).

calls or the detailed development of their consequences that need be altered. To the extent that the naturally occurring winter noise in VAI may occur because of meteorological instability, and to the extent that the meteorological instability may itself be sensitive to the processes that produce the presumed modulation, those processes may occasionally raise an incipient instability above, or suppress it below, the threshold level for rapid growth. The solar influence might then have a marked effect on the form (and not just the timing) of the weather, but we cannot conclude anything on the basis of the available data. The importance of the particular Sun–weather correlation discussed here may then lie not so much in the prediction of abnormal solar effects as in the improved prediction of normal meteorological processes.

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Conductivity and rectification in metal halide intervalence mixtures

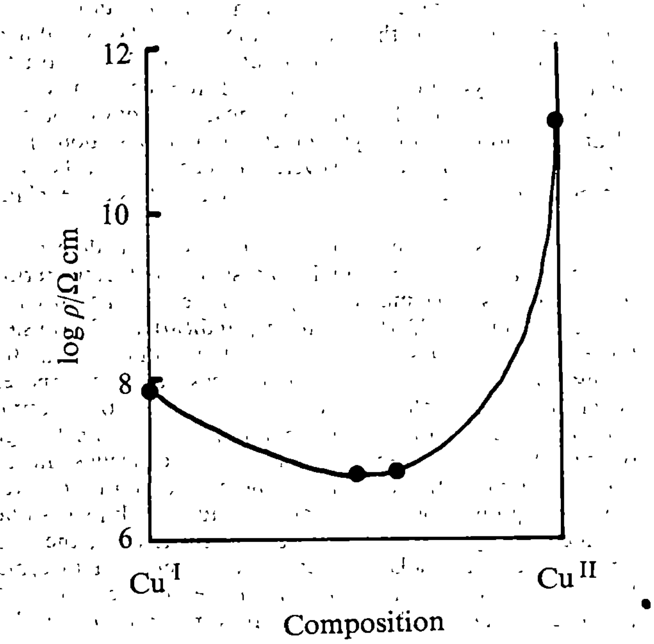
INTERVALENCE complexes contain two or more atoms of an element in different oxidation states; they have been collated and characterised as a group having properties peculiar to their valence structures[1]. Thus, the conductivity of a mixed-valence salt often exceeds that of either single-valence progenitor. We present here novel observations of just such effects in pressed-disk mixtures of copper(I) and copper(II) chlorides. The test is stringent because the (I) and (II) states have different geometries, tetrahedral and octahedral, and fall on the borderline of class 1–class 2 behaviour[1]. The disfavoured geometries from a Cu(I)–Cu(II) electron transfer event can, however, probably be mitigated as follows: disks pressed from ground powders can be used without thermal treatment, doubtless leaving single-state domains with dimensions close to those of the original particle sizes, and even though true intervalence can occur only at their boundaries, mixed and intermediate geometries are also likely. Figure 1 shows a tenfold enhancement of the Cu(I) value and ten thousand-fold enhancement of the Cu(II) value.

Since the conductivities are all low, in CuCl and the mixtures, electron hopping is the indicated mechanism, though anion migration has been proposed to explain the $CuCl_2$ process[2].

The two-centre electron-transfer process[3] has provided a basis for the interpretation and prediction of solid-state properties in the systems discussed here. We have assumed that chloride bridges between the various metal ions assist in lowering the barrier to hopping. We also aimed to adjust the effective Fermi levels in different regions by the introduction of oxidising or reducing chlorides—$FeCl_3$ and $SnCl_2$, respectively—themselves linked by chloride bridges to the intervalence host–matrix conducting system. Thus an $FeCl_3$-doped disk pressed to an $SnCl_2$-doped disk should generate excesses of the appropriate Cu valencies, which then annihilate at the contact leaving an effectively p–n junction; this proved to be the case.

Equimolar Analar white CuCl and brown $CuCl_2$ (dehydrated dihydrate) were ground together in a glovebox

Fig. 1 Log of resistivity/Ω cm against composition for CuCl–$CuCl_2$ disks.



containing dry nitrogen. An ordinary pellet press for infrared spectroscopy samples was used to obtain mottled, olive-green disks flecked with white and brown. A mixture containing 1% $SnCl_2$ was tamped down using a momentarily applied half-ton pressure, then a mixture containing 1% $FeCl_3$ was added as a further discrete layer, and a pressure of 12 ton was applied for several minutes. Disks 1.5 cm in diameter were released to a dry nitrogen atmosphere and ~ 0.2 cm^2 platinum contacts were silver-pasted on. For handling they were either sealed in wax or suspended in a desiccator with leads, and caged for measurements with shielded leads to an electrometer. Disk thicknesses were 1–3 mm.

With 1% doping, relative currents of 2.0 ($FeCl_3$ negative) and 1.6 ($SnCl_2$ negative) were observed. With 5% doping the relative values were 5.7 and 1.8, respectively. To establish that Sn^{4+} and Fe^{2+} space charges gave rise to the junction, we conjoined disks with 55:45 Cu(I)–(II) compositions. Nil rectification ensued.

Neither the actual conductivities nor the rectifications are themselves particularly impressive, but they illustrate far-reaching principles, from bottles of four reagents which just happened to be at hand. We have in mind applications to dithiolates[4] which, first, conduct relatively well as single components[5], second, exist in a wide variety of oxidation states[4] useful for intervalence mixing, third, show a wide range of reducing and oxidising ('donor' and 'acceptor') powers[4] useful for doping, and, in some solid state charge transfer adducts (D.R.R. *et al.*, ref. 7), show clear photovoltaism. The combination of these factors predicate good prospects for enhancing the efficiencies of photoelectric energy conversion. Finally, pressed disks (already established as satisfactory conducting systems[5,6]) must be cheaper than the fused systems used in technology.

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Precise determination of critical features in radiohalo-type coloration of biotite

HIGH precision techniques of photomicrography and densitometry are here used to investigate the coloration of artificially α-irradiated biotite. A computer simulated spherical radiation pattern is found to produce coloration peaks, contrary to the negative results of earlier investigators. A clearer understanding of the relationship between the usually defined α ranges and 'coloration ranges' is obtained as well as the elucidation of several other features of radiohaloes. In spite of more than a half a century of investigation, the parameters of α-induced radiation damage in biotite are still only crudely known. The principal parameter, the 'range' of the α particle, has been studied primarily by means of the uranium radiohalo and its close relatives (ref. 1; see also, refs 2 and 5). The coloration rings of these haloes lend themselves to direct, though somewhat inexact, measurements of the ranges of α particles of various energies. Early investigators also made use of measured α ranges in air and then converted these to ranges in biotite. Recently, Gentry[2,3] has made a substantial number of direct 'range' measurements in biotite and other minerals by bombardment with accelerator-produced α particles.

In spite of all previous work, a number of perplexing and basic concepts remain to be elucidated. We now report a series of precision measurements on biotite, from the Faraday pegmatite at Bancroft, Ontario, Canada[4], which was irradiated by α particles from the 5-MeV Van de Graaff accelerator at Wayne State University. Our investigations have explained a number of new features not previously recognised or, in some cases, recognised but not understood. We have been able to prove by direct irradiation techniques that a spherical, as distinct from a linear, radiation pattern will produce a series of coloration rings in biotite. Early experimenters[1] arrived at a puzzling negative result when they attempted by indirect means to prove this. We have also obtained the precise relationship between 'α range' as determined by the biotite coloration pattern and the true range. Moreover, we have made a high precision measurement of the range of the two most energetic uranium α particles, the 7.69-MeV and 6.00-MeV decays. A number of other puzzles have also been solved.

Blocks of biotite were polished to have a smooth face of ~1 cm by 2 cm perpendicular to the cleavage plane. The usual techniques of lens polishing worked well, but it was necessary to clamp the block tightly to keep the working face from fraying. The irregularity of the finished face was ⩽ 1 μm.

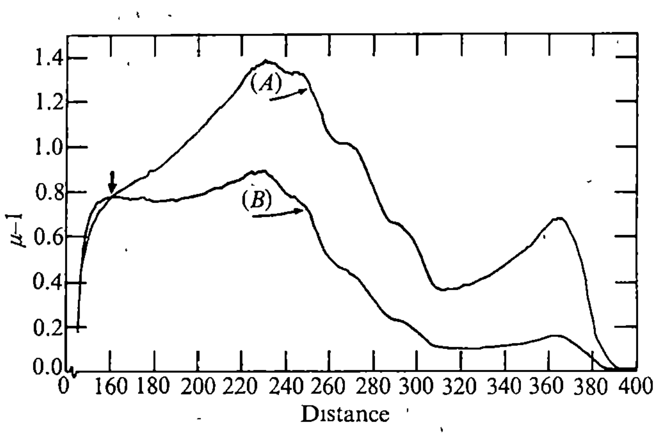

Fig. 1 *A*, The dimensionless optical absorption coefficient for α-irradiated biotite. *B*, The spherical absorption coefficient as derived from equation (3), arbitrarily normalised to *A* at the arrow. Notice the slight leftward shift of the curve *B* peaks due to the r^{-2} factor. The units along the ordinate are dimensionless since the absorption coefficient, μ, is expressed in units of the absorption coefficient for the natural (unirradiated) mica. The numbers along the abscissa are arbitrary, zero not being the mica edge but the start of the densitometer trace of the negative. The size of one abscissa unit is 0.147 μm. The first peak (4.2 MeV) occurs at ~ 230 on the abscissa.

While still clamped, the finished face was irradiated by an α beam striking it normally. The size of the beam spot was ~ 7 mm^2 and the intensity varied from 2×10^{-9} to 10^{-8} A as required. The Wayne State University accelerator produces highly monoenergetic particles, so the energy spread of several keV in the beam was insignificant for our purposes.

After irradiation the biotite was cleaved into flakes approximately 20 μm thick. For visual and photographic inspection of the thin sections, a Leitz Ortholux was used at a fixed magnification of 340.5, which we did not vary. After several unsuccessful photomicrographic results, we found that Kodak Contrast Process Pan 4155 film produced the best resolution with high contrast. In order to compensate for the nonlinear properties of this film (any film is, of course, nonlinear in some tone ranges), we calibrated it against a known photographic density wedge. All the results we report have been adjusted for the nonlinearity of the film.

Our photomicrographs were scanned by an Optronics Photoscan System P–1000 densitometer whose output was fed directly to magnetic tape. The tape was then run in conjunction with a program which reduces and analyses the raw data in several ways. We used the WSU IBM360/67 for all our work.

The key to understanding the densitometer tracings is to choose the appropriate physical variable for the computer output. This variable is the optical absorption coefficient of biotite, which is independent of the thickness of the specimen section. We express all our absorption coefficient results in units of the absorption coefficient for natural biotite, μ_N. If we designate the total optical absorption coefficient as μ_{tot}, we define our coefficient as $\mu \equiv \mu_{tot}/\mu_N$. Hence $\mu = 1$ is the value for the natural mica and α irradiation produces values for which $\mu > 1$.

In Fig. 1, curve *A* shows the plot of the absorption coefficient, μ, against distance from the edge of the mica section. We see five easily discernible peaks. (Actually there are 8 α-decay parent nuclei in the ^{238}U chain, with 11 significant α decays. Because the multiple α branches of the multi-branched nuclei are very close in energy, however, the literature has traditionally referred only to 8 α energies, of which only 5 can generally be resolved.) It is, however, critical to keep in mind that this is a plot of μ for a linear superposition of the eight energies. Haloes involve spherical superposition, which means that the radiation intensity drops by r^{-2} as the distance from the halo centre increases. The early experimenters[1], realising that halo darkening depended in some way on α-particle ionisation, attempted to use ionisation curves in air to produce a spherical halo pattern. By taking a linear superposition of eight ionisation curves, and then multiplying them by r^{-2}, they attempted to reproduce a halo. They had negative results. We now understand the reasons for their failure. They failed partly because: they used poor ionisation curves to start with; they falsely assumed the air–biotite conversion was independent of energy[5]; and they did not understand how true range related to 'coloration range'. Principally, however, they failed because ionisation is not the correct variable to display if one wishes to investigate coloration peaks. The correct variable, as stated above, is the absorption coefficient.

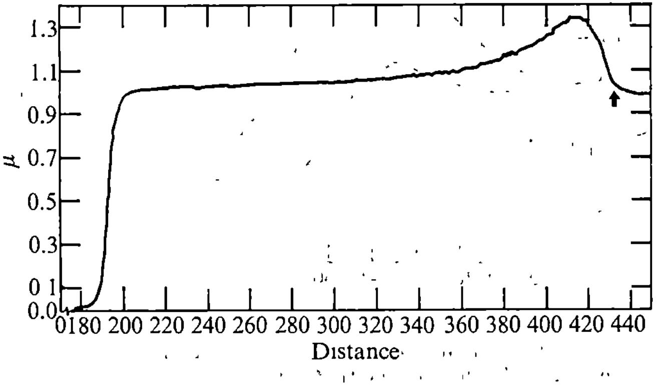

Fig. 2 The optical absorption coefficient curve for an α energy of 7.69 MeV. See Fig. 1 caption for an explanation of abscissa and ordinate values. The arrow points to the extrapolated ionisation range.

According to the elementary theory of F centres[6], the absorption coefficient will increase with radiation according to

$$\mu = \mu_{max}(1-\exp(-kd)) \qquad (1)$$

where μ_{max} is some characteristic maximum value, *k* is characteristic of the substance, and *d* is the particle dose. Deutsch *et al.*[7] experimentally verified in 1957 that such a curve holds very well for the α darkening of a wide variety of biotites. Of course, ionisation at any fixed point along the α path is proportional to the intensity of the primary radiation. From curve *A* in Fig. 1 we determine the dose which is

$$d = -\frac{1}{k}\ln\left(1-\frac{\mu}{\mu_{max}}\right) \qquad (2)$$

where μ_{max} has been determined for our biotite to be 2.87±0.02 by saturation dosing. Thus we see from equation (2) that the relationship between ionisation and darkening is simple only when the dose is small, and thus proportional to μ itself. To convert correctly the linear μ curve (*A*) in Fig. 1 into a simulated spherical optical absorption curve (*B*) we must multiply the dose by a factor r^{-2}, or, with r_0 an arbitrary normalisation point,

$$\mu_{sph} = \mu_{max}\left\{1-\exp\left[\left(\frac{r_0}{r}\right)^2 \ln\left(1-\frac{\mu}{\mu_{max}}\right)\right]\right\}$$

Our calculation took into account the fact that the above equation is not correct if artificial irradiation contributes darkening ionisation to an already naturally dark mica. The correct formula, used in our computations, is

$$\mu_{sph} = \mu_{max}\left\{1-\left(1-\frac{1}{\mu_{max}}\right)\exp\left[\left(\frac{r_0}{r}\right)^2 \ln\left(\frac{\mu_{max}-\mu}{\mu_{max}-1}\right)\right]\right\} \qquad (3)$$

We note that the peaks still exist in curve *B*, consistent with what is actually observed, and contrary to the results of previous investigators. Also, it is now obvious why Gentry found the 'ranges' in haloes somewhat shorter than those in artificially irradiated biotite. Equation (3) causes precisely this effect.

Figure 2 shows the μ curve for the single α energy of 7.69 MeV, at a low dose. Note the great similarity in shape to a typical ionisation curve[8,9]. This is because we have, as a first approximation to equation (1)

$$\mu \propto d \propto \text{ionisation} \qquad (4)$$

We can also see relationship of the peak or 'coloration range', to the usually defined ranges of the α particle. The extrapolated ionisation range, R_i (ref 8 and 9) is shown in Fig. 2. This range is very similar to the mean range, $\bar{R}$, but differs slightly from it.

Note that R_i is not the same as the 'coloration range', R_c, which actually corresponds to the peak of the ionisation curve. Nor is R_i the same as the frequently used 'threshold' or 'coloration extinction' range determined by the visual determination of the limit $\mu \rightarrow 1$, this latter range being dose dependent[2,3] whereas R_c and R_i are not. (Halo investigators from Joly on have, of course, been aware that the ionisation ranges of α particles in air gave, on conversion, mica ranges corresponding roughly to the outer foot of the coloration peak.) Since, in practice, coloration extinction is observed near, but above, the value $\mu = 1$, a 'threshold' range can be less or greater than an ionisation range depending on the dose. (To be absolutely precise R_i should be computed from the ionisation curve, whereas we used the μ curve of Fig. 2. When, however, $\mu \sim 1$, equation (4) shows that the choice of curves is immaterial.)

We have found the ranges to be

R_c(7.69 MeV) = 32.2±0.2 μm; R_c(6.00 MeV) = 21.4±0.2 μm
R_i(7.69 MeV) = 35.1±0.2 μm; R_i(6.00 MeV) = 23.7±0.2 μm

which we believe to be the most precise determinations of R_c and R_i yet given, as well as the first time a clear understanding of the different ranges has been achieved.

The precisely defined and carefully determined ranges given above are a necessary first step towards our further goal of comparing ancient α ranges with recent values in order to test the long term constancy of nature[4,5,10].

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'1/*f* noise' in music and speech

THE power spectrum $S(f)$, of many fluctuating physical variables, $V(t)$, is '$1/f$-like', varying as $f^{-\gamma}$ $(0.5\lesssim\gamma\lesssim1.5)$, over many decades of frequency (see ref. 1 for review). We have found that loudness fluctuations in music and speech, and pitch (melody) fluctuations in music exhibit $1/f$ power spectra.

$S(f)$ is related to the autocorrelation function $\langle V(t)\,V(t+\tau)\rangle$ by the Wiener–Khintchine relationships[2]. If $V(t)$ can be characterised by a single correlation time τ_c, $V(t)$ is correlated with $V(t+\tau)$ for $|\tau|<\tau_c$, and is independent of $V(t+\tau)$ for $|\tau|>\tau_c$. It can be shown that $S(f)$ is 'white' for frequencies $\ll 1/2\pi\tau_c$, and is a rapidly decreasing function of frequency (usually f^{-2}) for frequencies $\gg 1/2\pi\tau_c$. A quantity with a $1/f$ power spectrum cannot, therefore, be characterised by a single correlation time. In fact, the $1/f$ power spectrum implies some correlation in $V(t)$ over all time scales corresponding to the frequency range for which $S(f)$ is $1/f$-like[3]. In general, a negative slope for $S(f)$ implies some degree of correlation in $V(t)$ over time scales of roughly $1/2\pi f$. A steep slope implies a higher degree of correlation than a shallow slope.

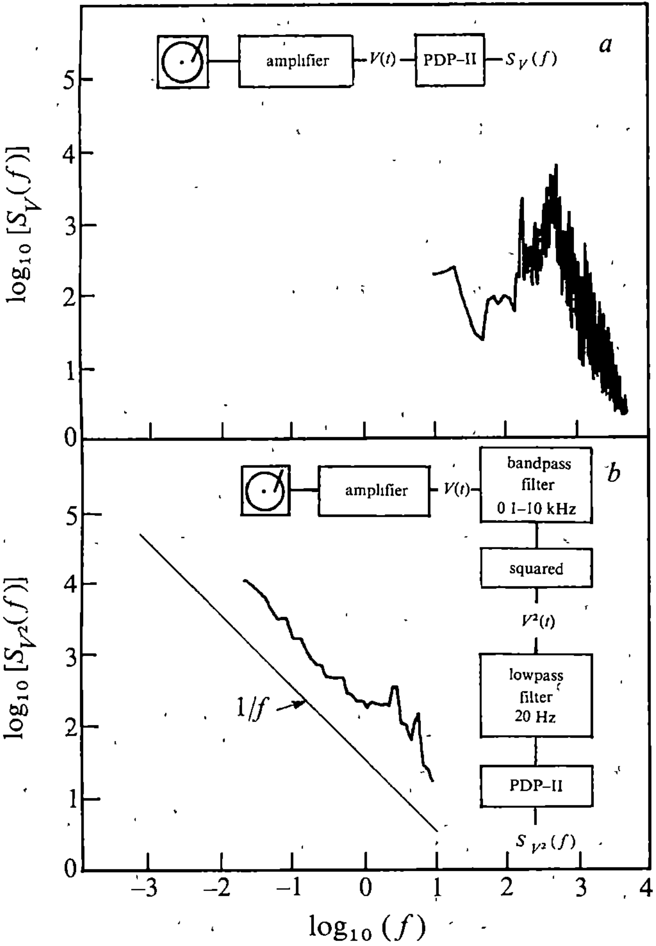

Fig. 1 Bach's 1st Brandenburg Concerto: *a*, $S_V(f)$ against f; *b*, $S_{V^2}(f)$ against f.

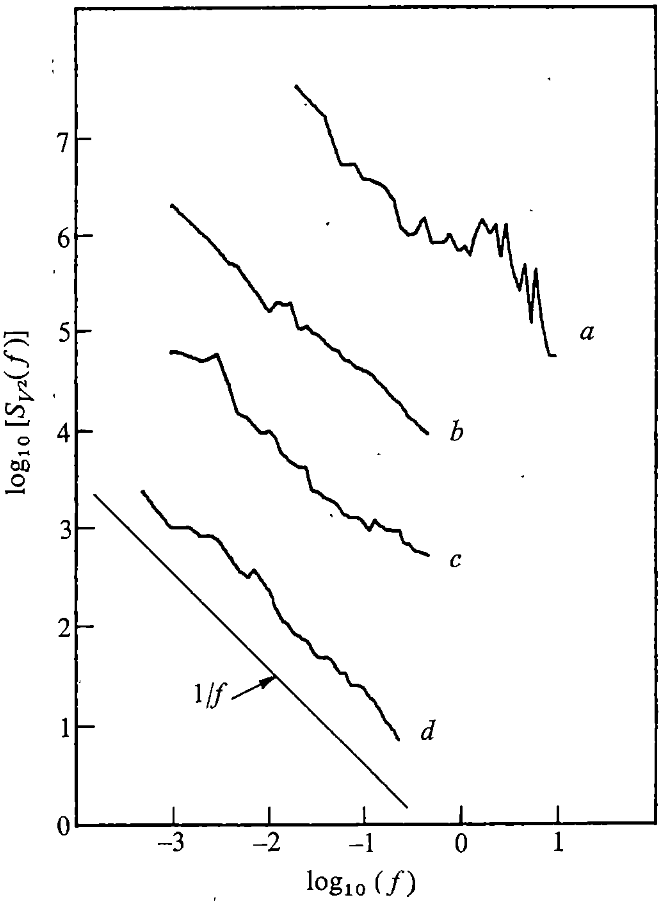

Fig. 2 Loudness fluctuation spectra, $S_{V^2}(f)$ against f for: *a*, Scott Joplin Piano Rags; *b*, classical radio station; *c*, rock station; *d*, news and talk station.

In our measurements on music and speech, the fluctuating quantity of interest was converted to a voltage whose power spectrum was measured by an interfaced PDP-11 computer using a fast Fourier transform algorithm. Our first measurements were on the output voltage, $V(t)$, of an audio amplifier. Figure 1*a* shows a log–log plot of the power spectrum, $S_V(f)$, of the audio signal from J. S. Bach's 1st Brandenburg Concerto averaged over the entire concerto. The spectrum consists of a series of sharp peaks in the frequency range 100 Hz–2 kHz corresponding to the individual notes in the concerto and, of course, is not $1/f$-like. Although this spectrum contains much useful information, our primary interest is in more slowly varying quantities.

One such quantity is the loudness of the music. The audio signal, $V(t)$, was amplified and passed through a bandpass filter in the range 100 Hz–10 kHz. The filter output was squared and the audio frequencies filtered off to give a slowly varying signal, $V^2(t)$, proportional to the instantaneous loudness of the music. The power spectrum of the loudness fluctuations of the 1st Brandenburg Concerto, $S_{V^2}(f)$, averaged over the concerto is shown in Fig. 1*b* on a log–log plot. Below 1 Hz the spectrum is $1/f$. The peaks between 1 and 10 Hz are due to the rhythmic structure of the music. Figure 2*a* shows the power spectrum of

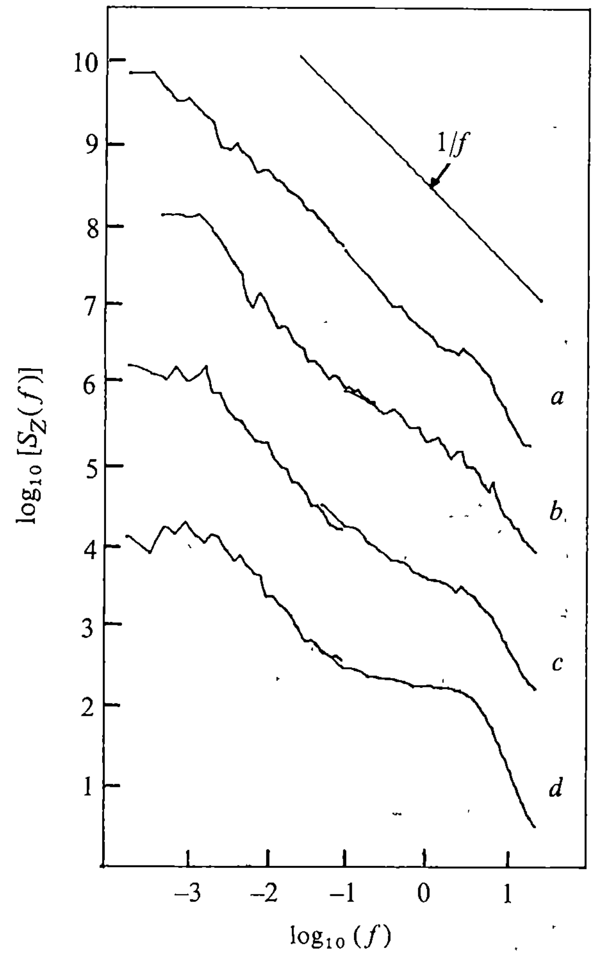

Fig. 3 Power spectra of pitch fluctuations, $S_Z(f)$ against f, for four radio stations: a, classical; b, jazz and blues; c, rock; d, news and talk.

loudness fluctuations for a recording of Scott Joplin Piano Rags averaged over the whole recording. Although this music has a more pronounced rhythm than the Brandenburg Concerto, and consequently has more structure in the spectrum between 1 and 10 Hz, the spectrum below 1 Hz is still 1/f-like.

To measure $S_{V^2}(f)$ down to even lower frequencies, we analysed the output from an a.m. radio. $S_{V^2}(f)$ was averaged over approximately 12 h, and thus included many musical selections as well as announcements and commercials. Figure 2b–d shows the loudness fluctuation spectra for three different radio stations. Figure 2b shows $S_{V^2}(f)$ for a classical station. The spectrum exhibits a smooth 1/f dependence. Figure 2c shows $S_{V^2}(f)$ for a rock station. The spectrum is 1/f-like above 2×10^{-3} Hz, and flattens for lower frequencies, indicating that the correlation of the loudness fluctuations does not extend over time scales longer than a single selection, roughly 100 s. Figure 2d shows $S_{V^2}(f)$ for a news and talk station, and is representative of $S_{V^2}(f)$ for speech. Once again the spectrum is 1/f-like. In Fig. 2b and d, $S_{V^2}(f)$ remains 1/f-like down to the lowest frequency measured, 5×10^{-4} Hz, implying correlations over time scales of at least 5 min. In the case of classical music this time is less than the average length of each composition.

Another slowly varying quantity in speech and music is the instantaneous pitch. A convenient means of measuring the pitch is by the rate, Z, of zero crossings of the audio signal, $V(t)$. For the case of music, $Z(t)$ roughly follows the melody. Figure 3 shows the power spectra of the rate of zero crossings, $S_Z(f)$, for four radio stations averaged over approximately 12 h. Figure 3a shows $S_Z(f)$ for a classical station. The power spectrum is closely 1/f. Figure 3b and c shows $S_Z(f)$ for a jazz and blues station and a rock station. Here the spectra are 1/f-like down to frequencies corresponding to the average selection length, and are flat at lower frequencies. Figure 3d, however, which shows $S_Z(f)$ for a news and talk station, exhibits a quite different spectrum. The spectrum is that of a quantity characterised by two correlation times: the average length of an individual speech sound, roughly 0.1 s, and the average length of time for which a given announcer talks, about 100 s. Communication, like most human activities, has correlations that extend over all time scales. For most musical selections the communication is through the melody and $S_Z(f)$ is 1/f-like. On the other hand, for normal English speech the pitches of the individual speech sounds are uncorrelated: the ideas are not directly related to the pitches of syllables but rather to the meanings attached to them. As a result, the pitch power spectrum for speech is 'white' for frequencies less than about 3 Hz, and falls as 1/f^2 for $f \gtrsim 3$ Hz. In fact, in Fig. 3a–c one observes these speech shoulders which become more pronounced as the vocal content of the music increases or the commercial interruptions become more frequent.

The observation of 1/f-like power spectra for various musical quantities also has implications for stochastic music composition. Most stochastic compositions are based on a random number generator (white noise source), which produces unrelated notes, or, on a low level Markov process, in which there is correlation over only a few successive notes. Neither of these techniques, however, approximates the 1/f spectrum and the long time correlations reported here in music. We have used independent 1/f noise sources in a simple algorithm to determine the duration (expressed as half, quarter, or eighth notes) and pitch (expressed in various standard scales) of successive notes of a melody. The music obtained by this method was judged by most listeners to be much more pleasing than that obtained using either a white noise source (which produced music that was 'too random') or a 1/f^2 noise source (which produced music that was 'too correlated'). Indeed, the sophistication of this '1/f music' (which was 'just right') extends far beyond what one might expect from such a simple algorithm, suggesting that a '1/f noise' (perhaps that in nerve membranes[4]?) may have an essential role in the creative process.

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Developmental changes in suckling of rat pups

Ontogenetic analysis of mammalian suckling provides a unique opportunity to observe the emergence of appetitive behaviour. Transitions in the nature of suckling may reveal progressive stages in the development of adult ingestive systems. Unfortunately, little information is available regarding the nature of suckling behaviour as it develops, and is eventually replaced by feeding and drinking, because it has proved difficult to separate the behaviour of the altricial infant from the contribution of the mother to the nursing dyad. We report here the developmental characteristics of, and changes in, the behaviour of suckling rats as they locate, apprehend and suckle the nipples of anaesthetised dams. This situation permits the detailed evaluation of rat pup behaviour in isolation from the active maternal role.

Even at birth, pups are capable of quickly apprehending and suckling the nipples of an anaesthetised dam. Suckling then undergoes two major transitions. The first, after day 10 *post partum*, suggests the emergence of a physiological control of suckling behaviour. The second, beginning after day 14, indicates a changing responsiveness to some characteristic of the mother's nipples or milk ejection.

Equithesin (Jensen–Salsbery Labs, 2 ml kg^{-1}) was the anaesthetic used. In the conditions reported here (lack of mammary engorgement and the presence of only a few pups), milk let-down with this type of anaesthetic was unlikely[1] Lack of milk ejection was further confirmed by comparing the weights (nearest 0.1 g) of pups before and after each test. For tests, the anaesthetised dam was laid on her side in a plastic trough (20-cm diameter, in a plastic tub 16 cm×28 cm×11 cm) with both nipple lines exposed (the lowest nipple 1 cm from the floor of the trough). Pups were gently placed in groups of three at the mother's side, perpendicular to her flank. The time required for each pup to attach (grasp and swallow) to a nipple was recorded. Also, general activity during the first 30 s was rated on a four-point scale and other behaviours (like rooting) were noted. At every test age, animals were exposed to the mother for three 5-min trials, each separated by a 5-min rest period; mean attachment latency for the three trials was used for analysis. Pups were tested with their own mothers, at several ages; tests of a litter were separated by at least 5 d (a period which was found not to interfere with normal growth). Between tests; pups remained with their mother and were not disturbed. Other pups were tested once only, at various ages, to confirm that specific testing experience did not contribute to attachment behaviour at the later ages.

Pups were tested in deprived and non-deprived conditions. Deprived pups were removed from the litter 22 h before testing, and housed in a warm moist incubator. Non-deprived pups were removed from the mother at anaesthetisation. Before, and between, tests axillary temperatures of both groups were kept at litter temperatures (32–36 °C) by placing pups on a warming tray.

Figure 1 presents the mean latencies of deprived and non-deprived pups to attach to the nipples of their anaesthetised mother. Even at the first test ages, pups were able to attach within the 5 min of the tests (41% at 0–1 d). During the following test intervals (until 8–10 d), the latencies of both deprived and non-deprived pups progessively decreased. This decrease reflects both an increase in the percentage of pups actually attaching, and a decrease in the latency of those that did attach. Attachment latencies for non-deprived pups were actually somewhat shorter than for deprived pups. Once attached, pups remained on the nipple for the duration of the test period despite lack of milk let-down. After 8–10 d, however, a marked transition occurred in the attachment behaviour of non-deprived pups. They shifted from rapid nipple attachment to attachment with long latencies or to non-attachment during the 5-min tests. Thus, before 8–10 d, attachment did not depend on deprivation state; both groups attached quickly and improved their latency to attach. After 8–10 d, attachment was dependent on deprivation (by 14–16 d the difference between deprived and non-deprived pups was statistically significant, $t=8.4$, d.f.$=48$, $P<0.001$, two-tailed). The change in attachment behaviour was not the result of a parallel change in general activity. Although a difference in activity appeared at 2–4 d, activity of the non-deprived pups remained essentially constant during the transition period [at 8–10 d mean activity rating of 1.8 (±0.13 s.e.m.), at 17–19 d mean activity of 1.7 (±0.09)] and by 20 d did not differ from that of deprived pups.

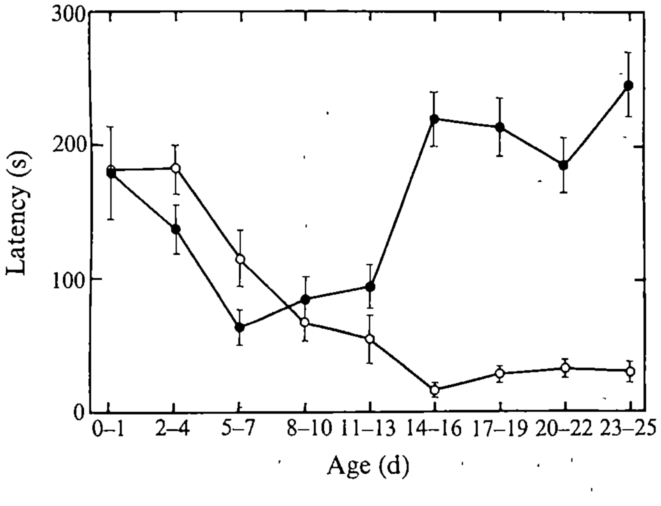

Fig. 1 Mean latency (±s.e.m.) of non-deprived (●) and 22-h deprived (○) rat pups to attach to nipples of their anaesthetised mother. Pups at 0–1 d were deprived for only 6 h. A pup's score was taken as the mean of its three 5-min tests. Pups were given a score of 300 s if they failed to attach. Number of subjects varied from 22–36 at each data point, with no more than six pups from the same litter. Pups were tested repeatedly, but not at each data point. The data presented here do not differ from that obtained from pups tested only once.

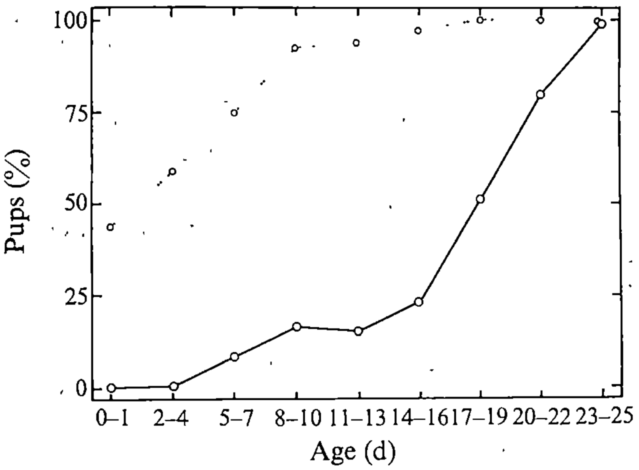

Fig 2 Percentage of deprived pups shifting nipples—after having attached. This is a mean of the percentage observed in the three 5-min tests. Data are presented only for deprived pups because, by the later ages, very few non-deprived pups attached. The percentage of attachments for these deprived pups is indicated by the dotted curve.

Starting at about day 14 a second change in pup behaviour became obvious. Deprived pups were observed to attach to a nipple, then 20–180 s later would shift to another nipple. The emergence of this behaviour in the sucklings is seen in Fig. 2. Before 14–16 d fewer than 25% of the pups shifted nipples after initial attachment. After 14–16 d, the percentage rapidly increased, until by 23–25 d virtually all pups shifted nipples at least once in each 5-min test. Actually most pups shifted repeatedly. A pup would attach to a nipple, suckle for a few moments and then reject it, only to attach quickly to another.

These findings contribute to a new understanding of aspects of rat suckling behaviour. First, the neonatal rat is capable of attaching and suckling on its own; the mother does not have to play an active role, even at parturition. The array of stimuli, thermal, tactile, and olfactory, that define the mother are sufficient to elicit and sustain suckling. This process in the young rat is independent of immediate milk delivery. In fact, we found that some pups 8 d old and younger will suck for at least 2 h without leaving the nipples of dams anaesthetised with Urethane (Lilly, 1 g kg^{-1}).

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Second, it is clear that suckling (as defined by the latency to attach to a nipple) is not under the control of nutritional events for the first 8–10 d after birth. Until that time, non-deprived rats apprehend a nipple at least as fast as those deprived of the dam for 22 hr. After that age, however, there is an abrupt change in behaviour, as non-deprived rats take considerably longer to suckle. This change strongly suggests the emergence of a physiological control of suckling.

Third, the development of nipple shifting indicates the nipple of the anaethetised dam is, after a certain age, no longer an adequate stimulus to sustain suckling in the rat pup. It is possible that pups come to view the nipple as a food source, and then respond in a way to maximise nutritional reward, leaving a nipple that is not producing milk or manipulating many nipples in order to stimulate milk ejection. It has also been noted that pups will leave a nipple just after a milk ejection, when gaining further milk from that nipple is unlikely[1].

Fourth, this preparation, which eliminates the active contribution of the mother from the nursing situation seems to be a reasonable way to isolate and analyse events that control suckling during ontogeny. Drewett, Statham and Wakerly[2] have recently presented an interesting analysis of the nipple attachments of 10-d-old rat pups hand-held by the experimenter at the nipples of anaesthetised mothers. They found that animals which had been deprived of food were quicker to attach to nipples than were non-deprived pups. Our data extend these findings to a situation where the pup itself is allowed to initiate the search and apprehension of the nipple, and demonstrate a developmental course for this apparent physiological control of suckling.

These data indicate the need for a reassessment of the traditional views of suckling. Suckling behaviour is more than a simple reflex that remains unmodified throughout development. It may prove as complex as the succeeding post-weaning ingestive behaviour of feeding and drinking, and an understanding of its development may contribute to our understanding of adult ingestion.

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Time resolution of acoustic signals by birds

SEVERAL workers have concluded that the response time of the avian ear is of the order of a few ms—far faster than that of the human ear. We have tested the ability of birds to discriminate between a single-click stimulus and a dual-click stimulus and found that this discrimination breaks down when the inter-click interval of the dual click is reduced to a few ms.

The evidence on which the previous conclusions were drawn is as follows (1) An unsuspected richness of detail emerges when recorded bird vocalisations are played back at slow speeds[1]. (2) An analysis of the constancy of reproduction of time intervals in songs of passerines suggests that time discrimination "is no greater than 0.5 ms and may well be considerably less"[2]. (3) The echolocating oil bird (*Steatornis caripensis*) produces pulses separated by silent intervals of 2–3 ms (ref. 3) and must presumably be able to discriminate echoes within this period. (4) Some birds which sing antiphonally demonstrate a precision of timing down to a few ms (ref. 4). (5) The basilar membrane of the bird ear is comparatively shorter and broader than that of the mammalian ear and the cochlear endolymph seems to be highly damped by the tegmentum vasculosum[5,6]. This implies that the response time of the avian ear is much shorter than that of the mammalian ear. (6) Electrophysiological responses in the cochlear nucleus of a number of species of birds show nearly 100% phase-locking with clicks reproduced at intervals down to 1–2.5 ms (ref. 7). The time resolution of the auditory neurones in the avian cochlear nucleus is by this measure superior to that of typical mammals.

Much of this evidence must be treated with great caution. Figures for the temporal resolution of the human ear vary greatly according to the method of testing, and should only be compared with those from birds tested in the same conditions. Greenewalt[2] measured the standard deviations of selected time intervals from repeated phrases of bird song. Equating acoustic performance with auditory discrimination, he suggested that these measures reflected the time discrimination of the avian ear. This would be true only if song production is controlled through auditory feedback. Auditory feedback, while necessary for song learning in some species, is not necessarily essential for the performance of adult song.[8] The constancy of reproduction of time intervals within adult song may then reflect the precision of 'read-out' from a central song template, perhaps influenced by non-auditory feedback loops, and not the time discrimination of the bird's ear. Further, the temporal patterning of song may, at the lower limits, depend on the response characteristics of syringeal muscles and not on reference to a memory. Responses in single auditory neurones cannot by themselves be taken as an accurate measure of perception. We have therefore sought direct behavioural evidence for temporal discrimination of sounds in birds.

Table 1. Discrimination between dual and single-click stimuli in three species of birds

Subject	Procedure	Discrimination threshold* (ms)
Bullfinch B♂2	Method of limits	6–3
Bullfinch B♂4	Method of constant stimuli	4–2
Bullfinch BJ2	Method of constant stimuli	4–2
Bullfinch B♂9	Method of constant stimuli	4–2
Greenfinch Gr♀2	Method of limits	Below 2
Greenfinch Gr♀3	Method of limits	6–2
Pigeon P♀1	Method of limits	10–2
Pigeon P♂1	Method of limits	10–2

For each subject discrimination between the dual click and a single click was significant ($P<0.05$) on the first stimulus value but not on the second. The difference between these two values reflects the number of stimulus values on which each subject was tested. Pigeons were tested on fewer stimulus values, and therefore their thresholds determined less precisely, than either the greenfinches or bullfinches.

*Inter-click interval of the dual click.

The discrimination between dual click stimuli and single click stimuli was investigated using operant conditioning Key pecking was rewarded by access to food. Birds were trained to peck only in the 7.5 s period following a train of dual-click stimuli (inter-click interval for training within the range 100–12 ms, train rate, 1 dual click per s, train duration, 5 s). The period between stimulus presentations was normally 25 s. Stimulus control was increased by having responses in the inter-stimulus period result in a 25 s delay in the onset of the next stimulus presentation. This training was followed by a series of daily 1-h tests in which dual and single click stimuli were randomised.

Responses following dual clicks were again rewarded with food and responses following single clicks resulted in a delay equivalent to that resulting from the period of food access.

The threshold for discrimination between dual and single-click stimuli was defined as the minimum inter-click interval for the dual click on which birds showed discrimination between the dual clicks and the single clicks. This threshold was determined for bullfinches (*Pyrrhula pyrrhula*) by three procedures. In the 'method of limits' the inter-click interval of the dual clicks was progressively decreased over a series of trials from 100 through 50, 25, 12, 6 to 3 ms. The 'method of constant stimuli' differed in that inter-click intervals of 12, 8, 6, 4 and 2 ms were randomised within the experimental series. The discrimination thresholds were found to be similar for both procedures (Table 1).

In the third method, the birds were trained to respond to dual clicks with a particular inter-click interval and the relative responsiveness to these and other clicks was then measured. Figure 1 shows the discrimination gradient obtained with this procedure for an adult male bullfinch. Generalisation is complete over the range 2–0 ms (the single click) and the threshold for discrimination of dual clicks from single clicks lies between 4 and 2 ms. This agrees with the results obtained by the other procedures.

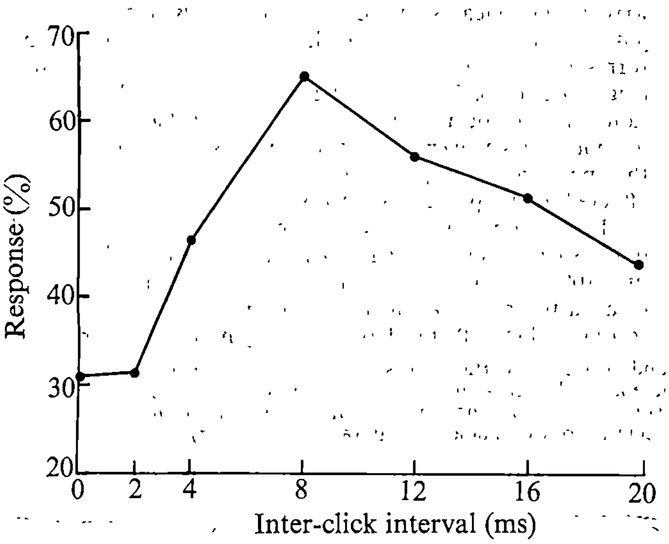

Fig. 1 Discrimination gradient over 15 generalisation tests on bullfinch B♂4. Each test contained 140 stimulus presentations, 20 on each of seven stimulus values. The click stimuli were presented in random order and the period between presentations was normally 25 s. If one or more key pecks were emitted during a given stimulus presentation then this was scored as a response. The responsiveness to each stimulus value was determined as the number of presentations on which responses occurred expressed as a percentage of the total presentations for that value. Stimulus control was increased by having responses in the inter-stimulus period result in a 25-s delay in the onset of the next stimulus presentation. Each test was preceded and followed by periods in which only stimuli with an inter-click interval of 8 ms were presented. Responses to these stimuli were rewarded with food and the periods were terminated after five rewarded responses. Responding during each test was maintained by rewarding responses to a predetermined five of the 20 stimuli with an inter-click interval of 8 ms.

We have also tested greenfinches (*Carduelis chloris*) and pigeons (*Columba livia*) and found their discrimination thresholds to be similar to those of the bullfinches (Table 1). Eight human subjects, tested under conditions similar to those experienced by the birds, reported that the two clicks were no longer heard as separate events when the interval between them was reduced to 12–50 ms. Two of the subjects additionally remarked that the composite 'fused' dual click was, however, still discernible from the single click down to inter-click intervals of 2–5 ms. Whether at the lower limits of discrimination the birds hear the two clicks of the dual-click stimulus as separate events or rely on other possible cues, for example, differences in perceived pitch between a 'fused' dual click and the single click, cannot be determined directly. It is, however, the absolute values for the discrimination thresholds which are important as these define the 'information content' of vocalisations[2]. In any vocalisation only that variation which occurs at suprathreshold level can be used by the bird as information in a communication system.

Rapidly executed frequency and amplitude modulations are common in bird song. The 'sequence calls' of bullfinches contain elements which show modulations with periods of 5–18 ms, and the modulation rates of these elements may be characteristic of particular individuals[9]. The discrimination gradient (Fig. 1) shows that bullfinches are able to discriminate, within the range 2–20 ms, between dual-click stimuli with inter-click intervals differing by only 2 ms. This suggests that information is encoded in the temporal patterning of the cells and is available to these birds. Such information may be of particular importance with regard to individual recognition in bullfinches.

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Inhibition of cortical evoked potentials and sensation by self-initiated movement in man

AN imposed, passive or externally paced displacement (EPD) of the index finger in man evokes brain potentials that differ in delay and waveform for postcentral, precentral and prefrontal areas[1]. Displacement so imposed leads to less accurate subjective estimation of limb position from that obtained by self initiated movement[2]. This behavioural observation as far as we know has not been neurophysiologically investigated with cortical recordings from the human brain. Thus we compared the cortical potentials following passive or externally paced displacement, to those related with a similar but self paced voluntary displacement (SPD) of the left index finger. Not only is self-initiated movement manipulative, however, it also subserves acquisition of sensory information. To examine this aspect of finger movement in a second series of experiments we studied the evoked responses and the subjective sensation to brief electrical stimuli applied to the left median nerve at the wrist or the index finger during SPD and compared them with similar responses elicited with the subject at rest.

Multichannel corticographic recordings have been obtained from chronically implanted gold electrodes from six subjects and with similar technique as previously described[1]. The finger displacement (of about 45°) was monitored by a movement transducer. The differentiated output of this transducer was used to trigger the PDP 12 computer which was programmed to average the cortical electrical activity preceding and following finger displacement. Somatosensory cortical evoked responses were elicited by electrical stimulation of the left median nerve of the wrist or the index finger. The stimulator was triggered either by the experimenter with the subject's finger at rest or by a Schmidt trigger activated by the movement during self paced displacement.

Consistently and for all subjects tested the area characteristic response following EPD has diminished, changed in wave form or disappeared completely when the subject himself was moving his finger. In Fig. 1 the evoked potentials from prefrontal, precentral and postcentral cortical areas following EPD can be seen on the left, and the cortical and electromyographic potentials related to similar but self paced displacement on the right. In this as for all subjects tested with SPD no response could be registered from the prefrontal areas, only a broad positivity developed in the postcentral areas and in the precentral areas a diminished amplitude response could be seen in the form of a negative peak.

These changes were not caused by the inherent variability of velocity of each self paced displacement, as averages of selected trials having similar time course showed similar reduction.

A gating action, movement related and exerted on the sensory pathways through their course to the cortex, can explain these results[3]. This hypothesis has been documented with data from animal experiments[3] but, until now, data obtained with scalp electrodes in man have been inconsistent between laboratories[4].

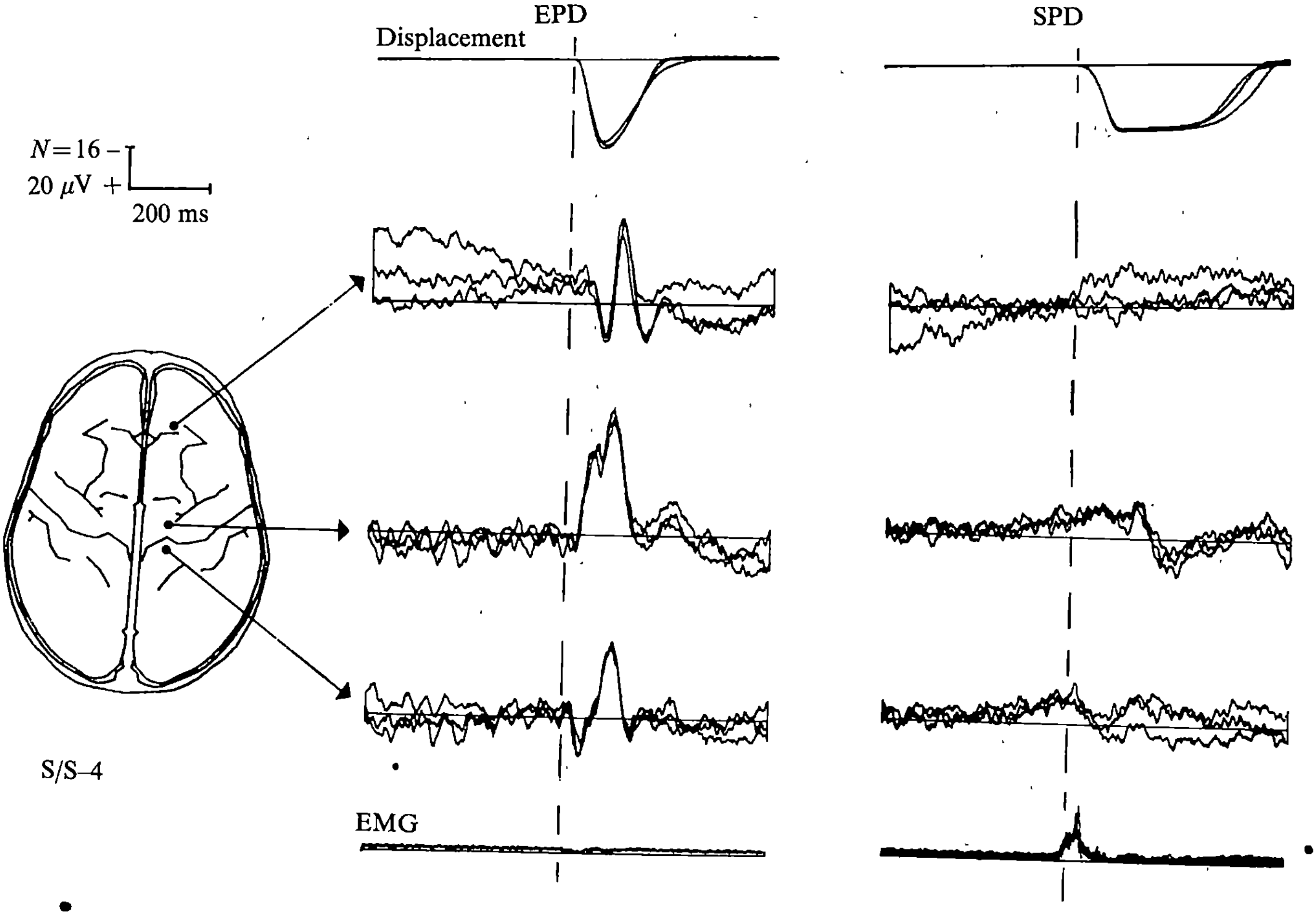
As was expected in SPD a sustained negativity developed in the motor-sensory areas before the initiation of the movement (Fig. 1). This is the Bereitschaft potential[5] or N_1 component[6] known to precede planned action. The negative peak of the precentral area response has been described previously as a "motor potential"[5] or N_2[6]. In our records this potential consistently developed only in the precentral cortex contralateral to the movement. Occasionally it preceded the beginning of the movement but never occurred before the beginning of the electromyogram related with the movement. The time from the peak of the electromyogram to the peak of this negativity was between 30 and 110 ms with most values falling between 30 and 50 msecs Contrary to previous suggestions[5,6] and in agreement with others[7], and our previous results[8] we consider this potential, which has been called a "motor potential", to be the first signal of reafferent activity resulting from the movement. We propose therefore, that N_2 is more appropriately called motor cortex potential (MCP). Our data thus suggest that movement related afferent activity directed towards the precentral cortex is less affected by gating action than when it ascends to other cortical areas.

The selective gating hypothesis has found additional support by studying the somatosensory evoked potentials from pre- and postcentral cortical areas during SPD.

Electrical stimulation of the left median nerve at the wrist evoked clear somatosensory potentials in the pre- and postcentral cortex when the limb was at rest. The waveforms of these potentials are different[9] for the two areas (Fig. 2*a* and *c*). The potentials evoked by similar stimulation timed to occur during self paced displacement of the left index finger were drastically diminished in amplitude in the postcentral region (*d*) but remained relatively unaffected at the precentral cortex (*b*).

These potentials in the postcentral areas were greatly diminished when the stimulus was presented during the first 100 ms of the movement. As the stimulus was further delayed the potentials increased in amplitude reaching their resting values 700 ms from the beginning. This recovery

Fig. 1 Superimposed evoked potentials from prefrontal, precentral and postcentral cortical areas due to left index finger displacement. The position of the recording cortical electrodes at the right hemisphere are indicated as dots in the brain plot. EPD, Responses due to externally paced displacement. SPD, Responses due to self paced displacement. Note that the general suppression during SPD of the potentials following EPD is less pronounced in the precentral cortex (middle cortical traces on the right).



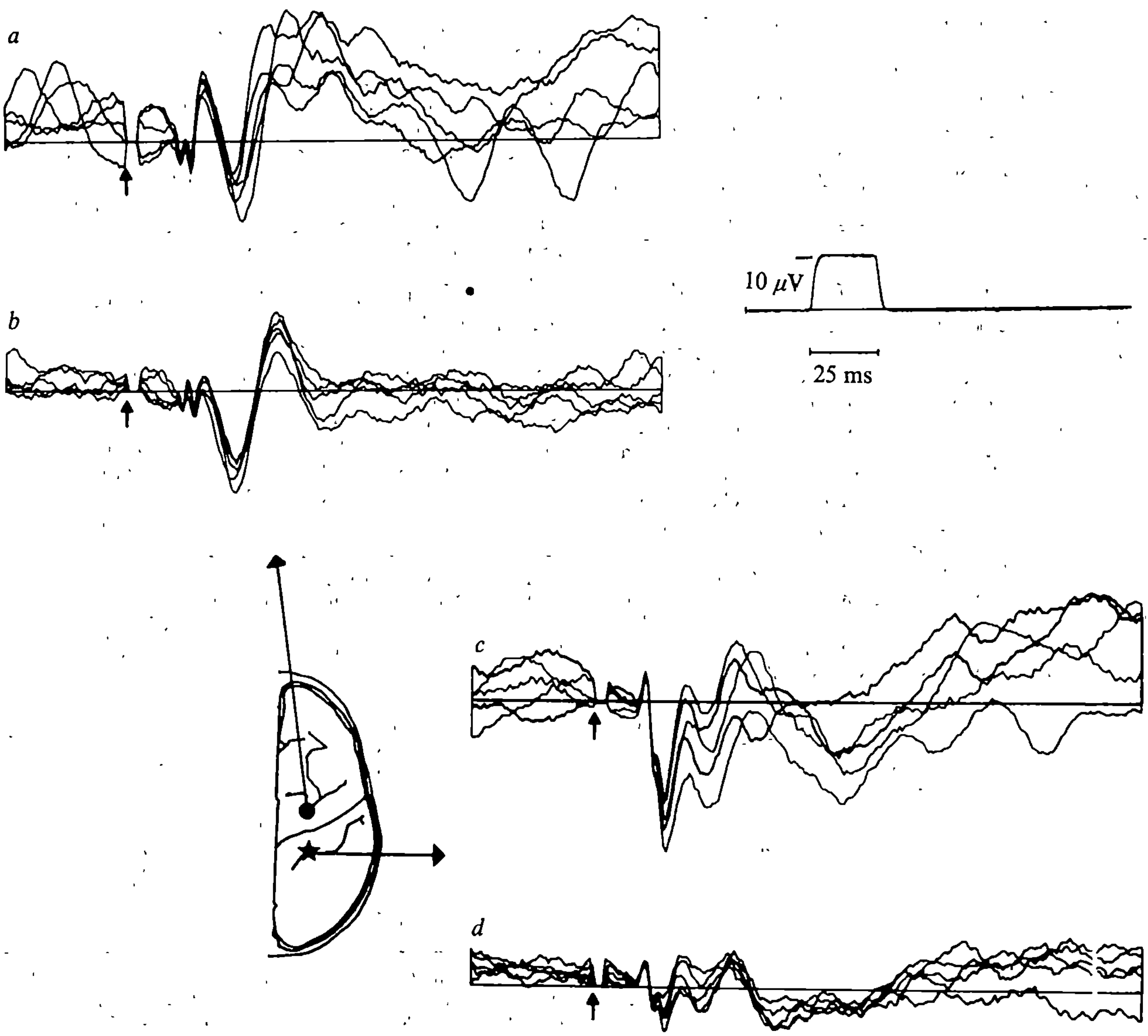


Fig. 2 Superimposed evoked potential to an electrical stimulus applied at the medium nerve at left wrist. Traces *c* and *d* from a right postcentral cortical electrode (asterisk in the brain plot). Traces *a* and *b* from a right precentral electrode (dot in the brain plot). Traces *a* and *c* have been simultaneously recorded from the two locations with the subject's finger at rest. Traces *b* and *d* have also been simultaneously recorded during a flexion movement of the left index finger around the metacarpophalangeal joint. Note the differential effect of the movement to the responses of the two areas; the postcentral one has been greatly reduced but the precentral response has retained its amplitude for the majority of the components.

occurred even if the finger was maintained in the displaced position. Both of these observations suggest that peripheral factors are not the cause of the diminished responses[10].

To clarify further the issue of gating and examine its possible behavioural manifestation the median nerve was stimulated and the subject's estimation of the stimulus strength has been determined with the finger at rest or moving. Ten subjects were asked to judge the intensity of three levels of electrical stimulation presented many times to the left index finger in random order when they moved the finger and when it was at rest. The subjective responses showed that all subjects experienced diminished sensation during movement. This diminution occurred only when the stimulated finger was being moved and not when other fingers or limbs were being moved.

Thus by subjective and electrophysiological data a centrifugal gating function is indicated to operate on the afferent activity resulting or occurring in the course of a movement. This gating action selectively affects the input to sensory and motor cortex the second one being less inhibited. Such reduced inhibition for sensory information related to movement and ascending towards the precentral motor cortex is in agreement with the theory of transcortical "reflex loops" proposed on the basis of data collected in animals[11] or from experimentally quantified performance in man[12]. The transcortical loop seems to operate through the precentral cortex as it is indicated by both the motor cortex potential (MCP) and the well preserved somatosensory evoked potential from precentral cortex during self-initiated movement. Consequently, the involvement of the primary efferent area in sensory function is at least double. It acts as an input gating mechanism for sensory information towards other cortical areas and as a selector of sensory information upon which prompt action is necessary. To what extent such properties can have clinical significance is too early to say, but it is pertinent to recall that shaking an affected limb is a familiar way of relieving pain after mild injury.

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Methylmercury and selenium in umbilical cords of inhabitants of the Minamata area

It has been established that selenium exerts a protective effect against the toxicity of methylmercury[1–5]. A study on higher marine mammals suggested that the antagonism between selenium and mercury may result in a marked accumulation of both elements in the organs of the animals[6], and a similar observation has been made in the laboratory[7]. In retrospect, this beneficial mechanism was operative, though incompletely, during the period when a wide area around the city of Minamata was polluted with methylmercury discharged from a factory involved in the production of acetaldehyde : the organs of men and cats who died from Minamata disease showed elevated levels of selenium as well as mercury[8,9]. Furthermore, the highest concentration of these substances was noted in the liver of an apparently healthy cat (Hg, 301 p.p.m.; Se, 89.7 p p.m.) caught in a village 10 miles north of Minamata[8,9]. We report here that the umbilical cords of the inhabitants of Minamata seem to reflect a chronological transition in methylmercury pollution but no corresponding shift in selenium levels.

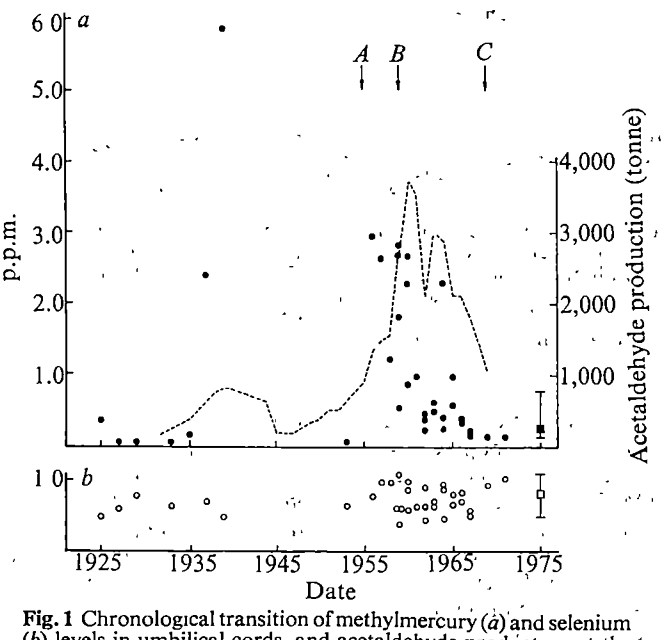

Fig. 1 Chronological transition of methylmercury (*a*) and selenium (*b*) levels in umbilical cords, and acetaldehyde production at the Minamata plant. The broken line represents monthly production of acetaldehyde. Methylmercury is a byproduct of this process. Concentrations of selenium and methylmercury are expressed on a dry weight basis *A*, Minamata disease publicly recorded. *B*, Methylmercury identified as causative agent of Minamata disease. *C*, Production of acetaldehyde terminated. ●, Methylmercury concentration. ○, Selenium concentration. Mean and s.d. ($N = 35$) were calculated as 0.73±0.19 p.p m. Congenital cases of Minamata disease are not included in this series. There is no particular level of methylmercury in umbilical cords at which a higher incidence of congenital Minamata disease[16]. ■, Average concentration of methylmercury in umbilical cords of inhabitants in Tokyo ($N = 24$); Maximum and minimum values are indicated by the bar. □, Average concentration of selenium in the umbilical cords of residents in Tokyo ($N = 24$, mean±s d. = 0 81±0.14 p.p.m.). The bar represents the maximum and minimum values. There was no significant difference between Minamata and Tokyo ($P > 0.5$).

The umbilical cords (~ 0.3 g) were obtained from the residents of the Minamata area (mostly fishermen) who had kept them in a dried state (a traditional custom in Japan is to use an infusion of the cord as a cure for serious illnesses). A piece (~ 5 cm long) excised at delivery is wrapped up in gauze and stored in a small wooden box; preservant is added only in exceptional cases. For the analysis of methylmercury we followed the method of Westöö[10], selenium was measured by the method of Watkinson[11].

The chronological transition of methylmercury and selenium levels in the cords is shown in Fig. 1. Although umbilical cord specimens during the period from 1941–1953 were unavailable, methylmercury levels in the cords seem to reflect two peak periods of acetaldehyde production : a minor peak in the late 1930s and early 1940s and a major one from the late 1950s until 1965 This relationship is more convincingly shown in the series of umbilical cords sampled from two fishery families (Table 1). This observation is in accord with, and expands, a report made by Fujiki *et al.*[12].

Table 1 Methylmercury and selenium concentrations in umbilical cords belonging to the members of two fishery families in Minamata

Family	Year of birth	Sex	Dry weight (p.p.m.) Methylmercury	Dry weight (p.p.m.) Selenium
A	1927	F	0.03	0.64
	1929	F	0.04	0.77
	1933	F	0.02	0.63
	1935	M	0.14	—
	1937	F	2 39	0.70
	1939	F	5.87	0.48
B	1953	M	0.04	0.63
	1956	M	2.94	0.76
	1959	F	2 82	0.60
	1962	M	0.22	0.43
	1965	M	0 97	0.63
	1966	M	0 38	0.70

Note that family A reflects the first (minor) peak of acetaldehyde production and the family B the second, and major, peak. The aetiology of Minamata disease was made public in 1960, and consumption of fish from Minamata Bay and the nearby sea greatly diminished, but was not completely stopped. Acetaldehyde production was terminated in 1967.

Two specimens from the late 1930s that showed remarkably high methylmercury levels belonged to the members of a family (family A in Table 1) who lived in a house located very close to the site where waste water from the factory was discharged. The first officially registered case of Minamata disease was put at 1953 in the initial study, but this was later changed to 1942, and the above finding supports the premise that cases of a disease with 'strange' neurological manifestations, noted before, and during those years, many of which were attributed to psychiatric origins, could have been caused by methylmercury[13]. In contrast to previous observations that selenium seems to accompany increased accumulation of mercury or methylmercury in some organs[6,7,9,14], this study shows that the selenium levels in the umbilical cords are fairly uniform throughout the observed years regardless of differences in location or time (Fig. 1), and that they are not influenced by methylmercury pollution. This discrepancy between the levels of mercury and selenium has also been noted for human hair (unpublished), and suggests a diversity in the underlying interaction of these elements.

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Investigation of cell lineage in bone using a chimaera of chick and quail embryonic tissue

MOST existing information about the nature of the stem cell(s) in bone that give rise to osteoblasts and osteoclasts is derived from autoradiographs prepared from tissues at various times after the administration of a pulse label of ^{3}H-thymidine[1]. These and related studies[2–5] suggest that a major bone cell precursor population is located immediately around developing bony rudiments and thus close to endosteal and periosteal bone surfaces. Unfortunately, the data obtained by tracing thymidine-labelled cells in bone is usually compromised by two technical problems. First, all cells in the S phase of the cell cycle, whether osteogenic or not, will incorporate ^{3}H-thymidine and appear labelled in autoradiographs. Second, since the isotope is available to dividing cells throughout the body, it is impossible to distinguish between labelled bone cells of local origin and those brought to the site by the blood vascular system. Bone does not represent a closed cellular system.

In the study reported here we followed the lineage of bone cells in a chimaera composed of Japanese quail limb and bone rudiments grown on the chorioallantoic membrane (CAM) of the chicken embryo. This system has the following advantages. First, the CAM provides an immunologically privileged site in which grafts are vascularised by blood vessels and blood cells with an origin different from their own[6,7]. Second, the distinct morphological differences between the interphase nuclei of the quail and those of the chick allow this organelle to be used as a permanent marker to determine the origin of cells in differentiated tissue at times long after the initial grafting procedure[8,9]. The interphase nuclei of most types of quail cells possess one or several large nucleolus-associated heterochromatic masses (Fig. 1*a*). The interphase nuclei of most chick cells, on the other hand, show a diffuse, somewhat stippled, distribution of heterochromatin (Fig. 1*b*).

Fertile Japanese quail (*Coturnix coturnix japonica*) and chicken (*Gallus domesticus*) embryos were raised to desired stages of development in a forced draft incubator at 37–38 °C. Embryos of both species were staged using the morphological criteria and numerical designations established for the chick by Hamburger and Hamilton[10]. Quail embryos at stages 23–35 (3–8 d incubation) were aseptically removed from their shells and transferred into sterile 0.9% NaCl solution. After removal of the attached extraembryonic membranes using a sterile technique, the fore and hind limbs were amputated. These tissues were then either subdivided into segments 1.5–3.0 mm long, or they were teased apart with watchmaker's forceps to isolate the cartilage models of the bones. The segments and rudiments were then transferred on to well vascularised portions of the CAMs of 7–11 d (stage 34–37) chick embryos through a window cut into the shell[11]. The graft-bearing eggs were resealed with sterile masking tape and incubated for an additional period of 7–8 d. It should be noted that the percentage of successful takes of cartilage models was significantly increased by placing the rudiments part of the way into a small rend or tear in the CAM. In such instances, the grafts increased 2–3 times in size during incubation and, on occasion, formed structures with strikingly normal external morphologies.

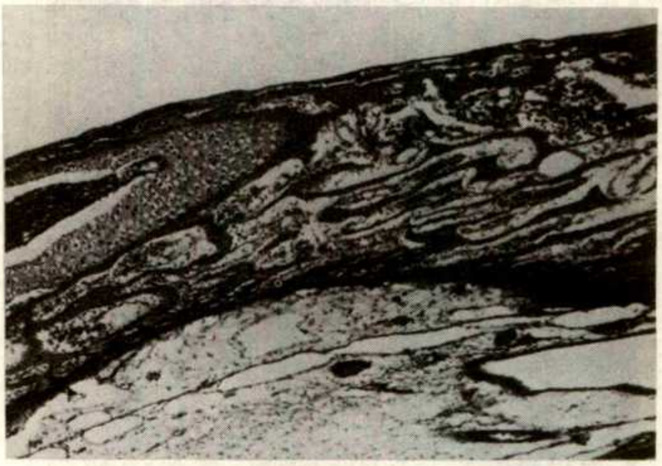

Fig. 2 Photomicrograph showing endosteal, periosteal and endochondral bone formation in a chimaeric graft. Haematoxylin and eosin. Magnification approximately ×20.

Grafts and control quail and chick tissues were collected

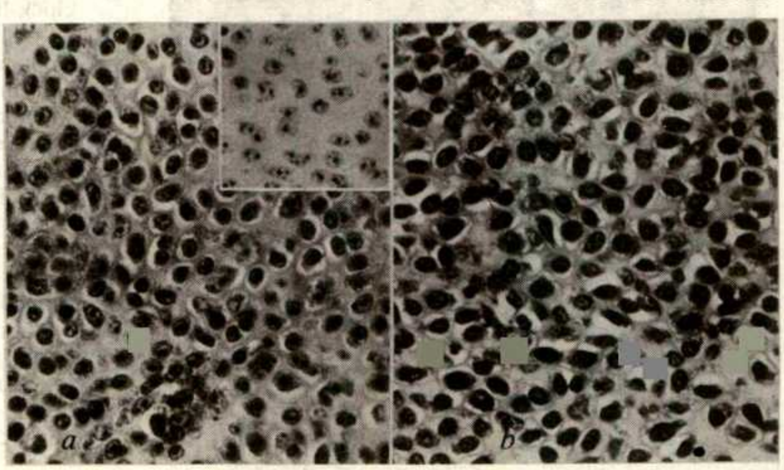

Fig. 1 H and E stained chondrocytes of the quail (*a*) and chick (*b*). The heterochromatic masses characteristic of quail interphase nuclei are even more apparent in Feulgen-stained tissue (inset). Magnification approximately ×208.

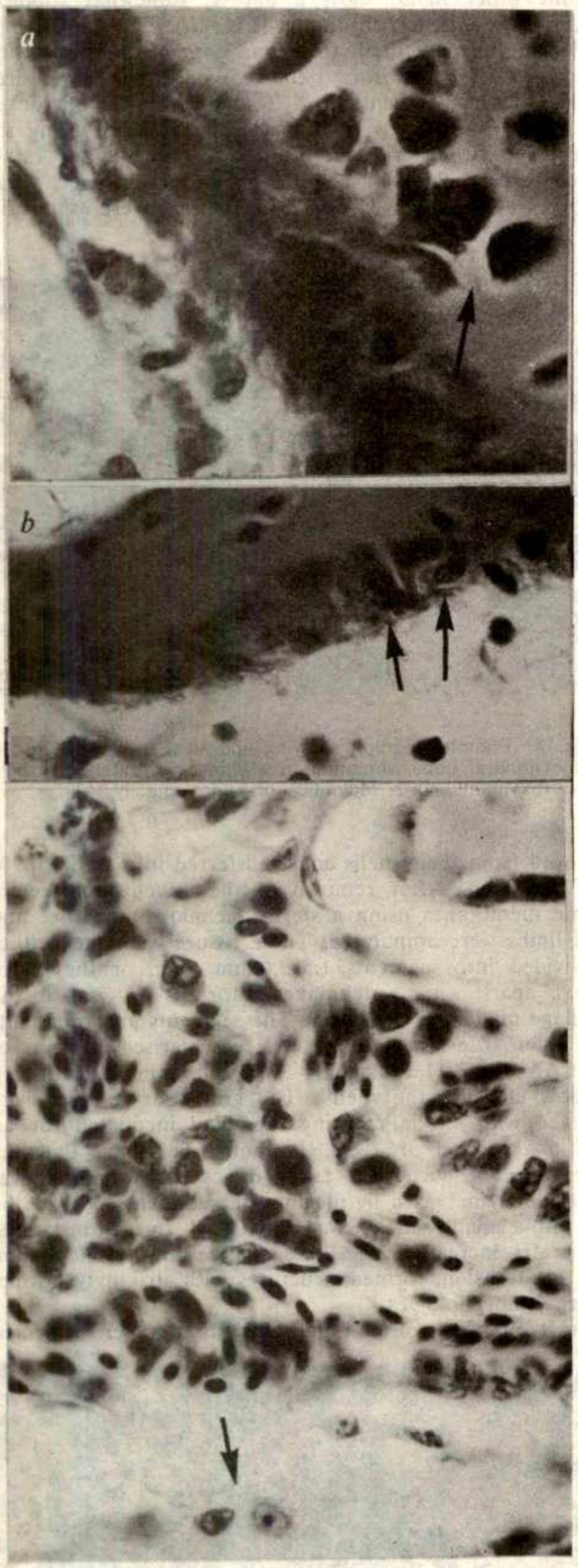

Fig. 3 *a* and *b*, Photomicrographs showing quail-like nuclei in osteoblasts (arrows) lining graft bone surfaces. *c*, The medullary cavity of the chimaeric graft, showing chick-like nuclei in a number of myelopoietic cells. Contrast these nuclei with the quail-like nuclei in the chondrocytes in the lower portion of the field (arrow). All haematoxylin and eosin. Magnifications approximately ×750 (*a*); ×300 (*b*); ×750 (*c*).

and fixed in 10% neutral formalin or Zenker's fluid and embedded in paraffin. Serial sections were then cut at 6–8 μm and stained with haematoxylin and eosin or by the Feulgen method.

Histologically, the grafts show skeletal development that is indistinguishable in major detail from the normal avian pattern. In favourable sections, endosteal and periosteal bone formation, cartilage hypertrophy and erosion, and myelopoiesis within expanding medullary cavities can all be observed (Fig. 2). In addition, some samples also show synovial joints at various stages of development.

Our observations on nuclear morphology suggest an interesting dichotomy in the activities of the chick and quail cells in the differentiation of the chimaeric graft. Most, if not all, of the cells involved in periosteal and endosteal bone formation have quail-like nuclei (Fig. 3*a* and *b*) indicating a donor (graft) origin for these cells. In contrast, most of the cells in the medullary cavities, including those involved in myelopoiesis, have chick-like nuclei, suggesting a host (haematogenous?) origin (Fig. 3*a*). There were, however, two interesting exceptions. First, cartilage cells in the epiphyses seemed to survive hypertrophy and were conspicuous in the metaphyses adjacent to the eroding cartilage matrix. Whether these cells are in fact viable and whether they can differentiate along osteogenic pathways as suggested by Holtrup[12] and others[13] is at present under investigation. Second, multinucleated osteoclasts and chondroclasts seemed to be of three types: those with chick-like nuclei, those with quail-like nuclei and those with an admixture of chick and quail-type nuclei. (Fig. 4*a* and *b*). The osteoclasts and chondroclasts with chick-like nuclei were the predominant type.

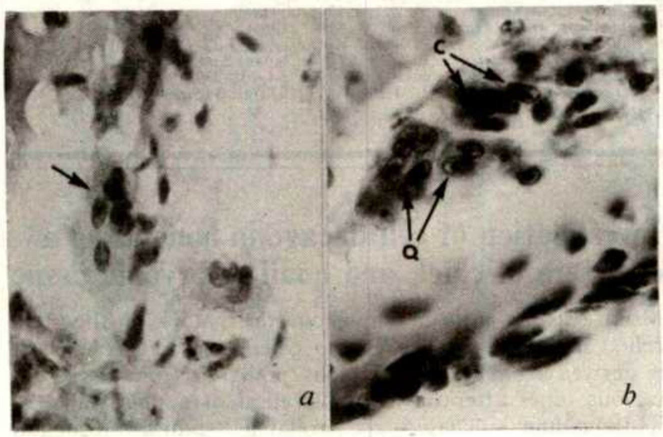

Fig. 4 Photomicrographs showing the nuclear composition of some osteoclasts in chimaeric tissue. Note that there are only two quail-like nuclei (arrow) in the osteoclasts in *a*, whereas in *b* a number of nuclei of both types (Q, quail-like; C, chick-like) can be seen. Feulgen (*a*) and haematoxylin and eosin (*b*). Magnification approximately ×470.

It should be noted that not all the cell types in the chimaeric bones expressed the distinctive species-related pattern of nuclear morphology equally well. The problem was most acute for the cells in the medullary spaces, either because of the heterochromatisation of the nucleus (as in maturing erythrocytes) or because, in some cells, the nucleoli were less compact and distinctive (osteoclasts/chondroclasts, myelocytes). The species origin of osteoblasts, osteocytes, chondrocytes and fibroblasts could almost always be established with confidence in well fixed and well stained preparations.

These observations support the concept that most osteoclasts are derived from mononucleated haematogenous cells such as phagocytes and monocytes[15-20]. They also confirm[4,21,22] that some osteoclasts arise from the "fusion" of bone cells *in situ*. We found no evidence, however, that osteoclasts can disaggregate to form osteoblasts directly or indirectly through dedifferentiation to precursor cells. The percentage of osteocytes in both chimaeric and native quail bone that had quail-like nuclei was identical (~90%). It would seem, therefore, that few (blood-borne) chick-type osteoclast nuclei in this bone modelling system have the capacity to form osteogenic cells. The survival and/or death of osteoclast and chondroclastic cells in bone thus remains an open question. Confirmatory studies using tissues labelled with ^{3}H-thymidine and immunohistology are in progress.

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Stimulation of *in vitro* somite chondrogenesis by procollagen and collagen

THE embryonic notochord stimulates or induces the formation of vertebral cartilage by embryonic somites[1,2]. Evidence indicates that proteoglycans produced by the notochord are involved in mediating the conversion of somitic sclerotomal cells into cartilage. During its interaction with somites, the notochord synthesises and secretes proteoglycans (and collagen) which initially accumulate in the perinotochordal sheath and subsequently diffuse among adjacent somitic sclerotomal cells just before the differentiation of the cells into cartilage[3]. Extracellular proteoglycan aggregate (consisting predominantly of proteochondroitin 4- and 6-sulphate) greatly stimulates *in vitro* somite chondrogenesis in the absence of notochord[4]. Notochords from which proteoglycans of the perinotochordal sheath have been selectively removed are impaired in their ability to support chondrogenesis but reacquire their ability to stimulate chondrogenesis concomitant with their reacquisition of perinotochordal proteoglycans[5,6].

Although these results implicate proteoglycans produced by the notochord in the control of somite chondrogenesis, several observations suggest that collagen produced by the notochord may also be involved[3]. Collagen synthesised by the notochord consists only of α1 chains and seems to have an amino acid composition identical to type II cartilage collagen[7,8] In addition, notochords from which both the collagen and proteoglycans of the perinotochordal sheath have been removed are more impaired in their ability to support chondrogenesis than notochords from which proteoglycans have been selectively removed[5,6]. Finally, collagen, as well as proteoglycans, has been directly implicated in one other tissue interaction, that between lens and corneal epithelium[9,10].

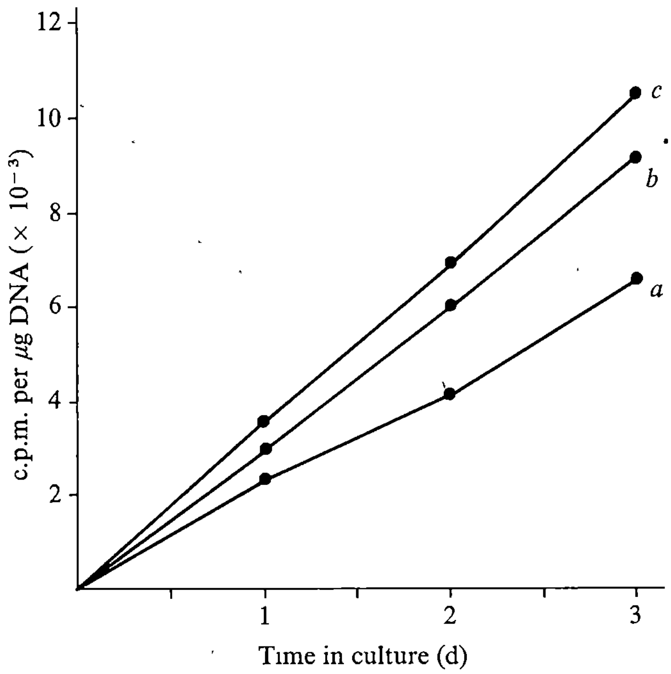

Fig. 1 Accumulation of ^{35}S-sulphate-labelled glycosaminoglycans (GAG) by control somite explants (*a*) and by explants supplied with exogenous type I (*b*) and teratoma (*c*) procollagen. GAGs were extracted from somite explants continuously exposed to 5.0 μCi ml^{-1} of $H_2{}^{35}SO_4$ (carrier free, New England Nuclear) described in ref. 5. DNA was determined by a micromodification of the procedures of Abraham *et al.*[21] and Richards[22]. The experiment was repeated five times and the accumulation of ^{35}S-sulphate-labelled GAG by explants in the presence of type I procollagen ranged between 30% and 50% above the controls by the third day of culture, whereas the accumulation in the presence of teratoma procollagen ranged between 60 and 65% above the controls.

We have investigated the effect of collagen on *in vitro* somite chondrogenesis. In addition, since it has been established that collagen is initially synthesised and secreted in a soluble precursor form (procollagen), it was of interest to test the effect of procollagen. Further, the extensive solubility of procollagen in physiological conditions enabled us to compare different procollagen types at equivalent concentrations on their ability to stimulate chondrogenesis. We have found that several distinct molecular species of procollagen and collagen do indeed stimulate *in vitro* somite chondrogenesis. Further, our results suggest that certain types of procollagen and collagen are more effective than others in promoting chondrogenesis. Particularly effective in promoting chondrogenesis is type II collagen, which is the type of collagen synthesised and secreted by the notochord during its interaction with somites.

Somites were dissected from stage 17 embryos[11] of White Leghorn chicks (see ref. 12). Explants consisting of 8–10 somites were cultured on nutrient agar[13] and fed with F12X medium containing 20% foetal calf serum, and were examined daily for visual signs of chondrogenesis (see ref 13). To determine the effect of exogenous procollagen on chondrogenesis, the feeding medium was supplemented with 200 μg ml^{-1} of procollagen. Type I procollagen was purified from the medium of cultured human diploid skin fibroblasts (see ref. 14). These molecules have a chain composition of $[\mathrm{Pro}\,\alpha1(\mathrm{I})]_2$ $\mathrm{Pro}\,\alpha2$ and demonstrate an amino acid composition comparable to that of type I procollagens purified from other sources[15]. Procollagen was also purified from the medium of a clonal line of mouse teratoma cells.

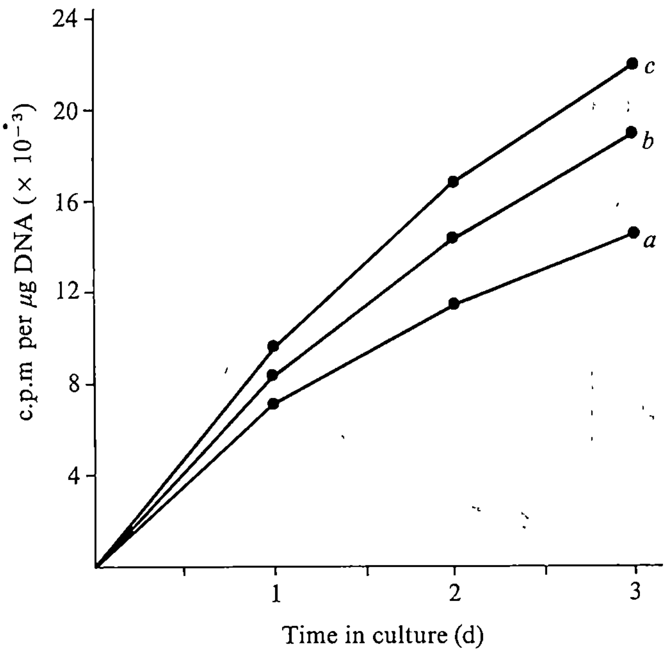

Fig. 2 Accumulation of ^{14}C-glycine-labelled collagen by control somite explants (*a*) and by explants supplied with exogenous type I (*b*) and teratoma (*b*) procollagen. Collagen synthesis was assayed by the incorporation of ^{14}C-glycine into collagenase-sensitive materials utilising a protease-free collagenase (see refs 19 and 20). The experiment was repeated three times and the accumulation of ^{14}C-glycine-labelled collagen by explants in the presence of type I procollagen ranged between 30 and 35% above the controls by the third day of culture, whereas the accumulation in response to teratoma procollagen ranged between 46 and 55% above the controls.

This teratoma procollagen seems to be of a unique type since it consists only of Pro α1 chains and has an amino acid composition that is distinctly different from all other known collagens and procollagens having only α1 or Pro α1 chains in the molecule (R. L. Church, in preparation). In addition, somite explants were cultured on Millipore filters (type HA, 0.45 μm pore size) or on Millipore filters on which collagen substrates were prepared. Collagen substrates were made by preparing collagen gels on Millipore filters (see ref. 16) and concentrating the collagen in the gels by drying (see ref. 9). Purified type II collagen from lathyritic chick sternal cartilage was prepared by differential salt fractionation[17]. Purified type I collagen from calf skin was prepared as described in ref. 18.

Repeated visual observations indicated that in the presence of exogenous Type I or teratoma procollagen there was a consistent increase in the amount of hyaline cartilage matrix formed by somites. To confirm these visual observations of enhanced chondrogenesis, we studied the accumulation of sulphated glycosaminoglycans (GAG) and collagen, the major constituents of cartilage matrix, in response to exogenous procollagen.

The accumulation of ^{35}S-sulphate-labelled GAG by somite explants cultured in medium supplemented with type I and teratoma procollagen is shown in Fig. 1. By the third day of culture, somites cultured in the presence of type I procollagen accumulated approximately 40% more sulphated GAG than controls. This experiment has been repeated five times and the accumulation of sulphated GAG by explants in the presence of extracellular type I procollagen ranged between 30% and 50% above controls. In contrast, somites cultured in the presence of extracellular teratoma procollagen consistently accumulated 60–65% more sulphated GAG than controls (Fig. 1). These results indicate that although both types of procollagen are stimulatory, teratoma procollagen is more effective than type I.

Figure 2 demonstrates the accumulation of ^{14}C-glycine-labelled collagen by somites in response to extracellular Type I and teratoma procollagen. Explants in the presence of type I consistently accumulated 30–35% more collagen than the controls, whereas those in the presence of teratoma procollagen consistently accumulated approximately 50% more collagen than the controls.

These results indicate that both types of procollagen stimulate sulphated GAG and collagen accumulation by somites, an observation that correlates well with the consistent increase in the amount of cartilage matrix formed in response to each type of procollagen. Further, they indicate that although both types of procollagen are stimulatory, teratoma procollagen is a more effective stimulator than type I. The stimulatory effect of procollagen on sulphated GAG and collagen accumulation by somite explants seems to be specific for these matrix components, since type I procollagen had no effect on the incorporation of ^{14}C-glycine into non-collagen protein, and teratoma procollagen increased the accumulation of non-collagen protein by only 5%. In addition, only 4–5% of the total labelled GAG and collagen accumulated by explants supplemented with type I or teratoma procollagen was found in the medium of the cultures, whereas 8–9% of the total labelled material was found in the medium of the control explants.

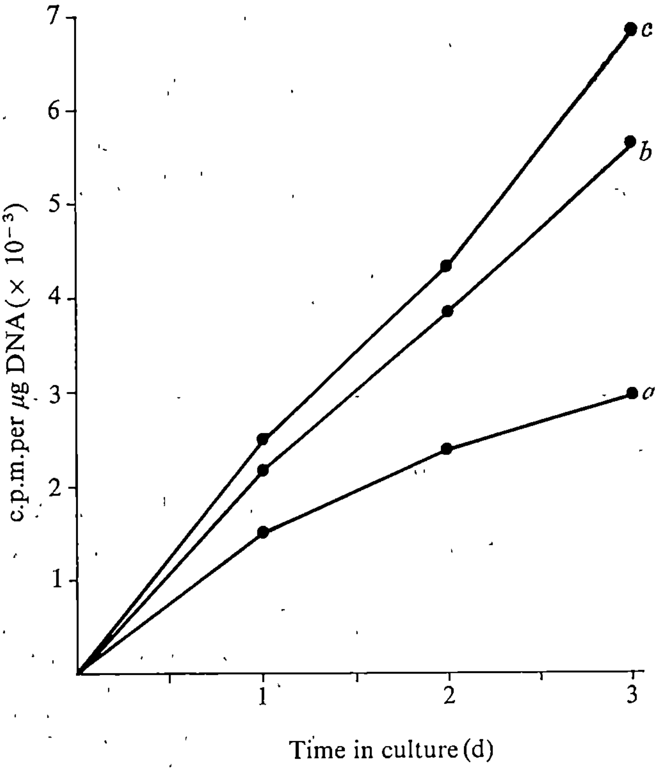

Fig. 3 Accumulation of ^{35}S-sulphate-labelled glycosaminoglycans (GAG) by control somite explants cultured on Millipore filters (*a*) and by explants cultured on type I (*b*) or type II (*c*) collagen substrates. The experiment was repeated four times and the accumulation of ^{35}S-sulphate-labelled GAG by explants cultured on type I collagen substrates ranged between 1.95 and 2.05 times greater than the controls by the third day of culture, whereas the accumulation on type II substrates ranged between 2.4 and 2.5 times greater than the controls.

This suggests that procollagen not only stimulates the total accumulation of matrix components by somite explants, but that it also enhances the retention of these components by the tissue. Finally, neither type I nor teratoma procollagen seems to enhance the survival or viability of somite explants, since there is no difference in the total DNA content of control or experimental explants during any period of culture.

The response of somites cultured on purified type I or type II collagen substrates prepared on Millipore filters was compared with the response of somites cultured on Millipore filters alone or on Millipore filters coated with bovine serum albumin.

On collagen substrates there was a striking increase in the amount of hyaline cartilage matrix detectable visually with the dissecting microscope and histologically. A detailed light microscopic and ultrastructural analysis of the enhanced chondrogenic response of somites to collagen substrates will be published elsewhere. The visual observations correlate well with the biochemical response of somites to collagen substrates as shown in Figs 3 and 4.

Figure 3 compares the accumulation of ^{35}S-sulphate-labelled GAG by somites cultured on type I and II collagen substrates with the accumulation by controls cultured on Millipore filters. By the third day of culture, there was a consistent twofold increase in the amount of sulphated GAG accumulated on type I substrates and a 2.4- to 2.5-fold stimulation of type II substrates.

Similarly, approximately 75% more ^{3}H-proline-labelled collagen was accumulated by somites on type I substrates than by controls, and twice as much collagen was accumulated on type II substrates (Fig. 4).

The enhanced accumulation of the components of cartilage matrix by somites on collagen substrates seems to be relatively specific, since the accumulation of ^{3}H-proline-labelled non-collagen protein is increased by only 24% on type I substrates and by only 31% on type II substrates. The slightly enhanced accumulation of non-collagen protein on collagen substrates undoubtedly reflects to some extent the enhanced accumulation of the protein core of sulphated GAG (proteoglycan). Further, there is an enhanced retention of matrix components by the tissue on collagen substrates, since only approximately 4% of the total labelled GAG and collagen accumulated by explants on collagen substrates is liberated to the medium, whereas approximately 10% is found in the medium of control cultures. In addition, collagen substrates do not affect the viability of explants, since there is no difference in the total DNA content of control and experimental explants during any period of culture.

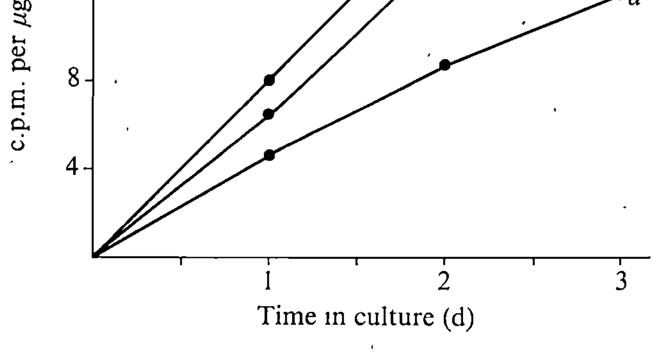

Fig. 4 Accumulation of ^{3}H-proline-labelled collagen by control somite explants cultured on Millipore filters (*a*) and by explants cultured on type I (*b*) or type II (*c*) collagen substrates. The experiment was repeated four times and the accumulation of ^{3}H-proline-labelled collagen by explants cultured on type I collagen substrates ranged between 1.75 and 1.81 times greater than the controls by the third day of culture, whereas the accumulation on type II substrates ranged between 1.95 and 2.07 times greater than the controls.

We were unable to test the effect of type II procollagen on somite chondrogenesis, since we do not have an adequate supply of purified Type II procollagen. That is, however, an important experiment for future investigation as the indications are that type II is the type of collagen synthesised and secreted by the notochord during its interaction with somites[7,8].

We did, however, find that purified type II collagen substrates caused a striking stimulation of *in vitro* somite chondrogenesis. Furthermore, type II substrates were consistently more effective in promoting chondrogenesis than type I collagen substrates. We cannot, however, be certain that somites have access to equivalent amounts of collagen on each type of substrate. Although we have made every effort to ensure that the substrates of the two types of collagen were prepared from collagen solutions of equivalent concentrations, it is difficult to control accurately the amount of collagen interacting with tissues using insoluble collagen substrates, because of potential differences in the polymerisation properties of the two types of collagen or potential differences in the thickness of the two substrates[9]. Nevertheless, the fact that we have never done an experiment in which type II collagen did not cause a greater stimulation than type I suggests that type II collagen may indeed be more effective than type I in promoting chondrogenesis. We should be able to answer this question about collagen type specificity more clearly when we have examined the effect of soluble type II procollagen on chondrongenesis

An important consideration in these experiments is the purity of the procollagen and collagen preparations used to promote chondrongenesis. Although it is difficult to rule out absolutely the possibility that a trace amount of non-collagen material is present in any purified collagen preparation, the purity of all of the procollagen and collagen preparations used in these experiments has been established by a number of rigid criteria[14,17,18].

The mechanism by which extracellular matrix materials stimulate somite chondrongenesis is not known. It has been suggested that extracellular proteoglycans exert their effect on somite chondrongenesis by interacting with components of the somite cell surface[4,5]. Similarly, we propose that a likely site of action of collagen is the somite cell surface. The fact that completely insoluble collagen substrates stimulate chondrongenesis is strong evidence that collagen is exerting its regulatory effect by interacting with the cell surface.

Regardless of its mechanism of action, the results of the present investigation indicate that collagen, as well as proteoglycans, is involved in the control of somite chondrogenesis and that these two classes of extracellular matrix materials may synergistically mediate the conversion of somite sclerotomal cells into cartilage.

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Early-labelled haem in erythroid and hepatic cells

SEVERAL lines of evidence point to the existence of a rapidly synthesised haem fraction which is unrelated to any of the known haemoproteins. Much of this evidence has come from studies of the early-labelled fraction of bilirubin which, unlike most bilirubin produced in normal conditions, is derived from sources other than haemoglobin in senescent red cells[1,2]. As measured with ^{14}C-glycine, the early bilirubin fraction consists of both a rapid and a slow component[3,4]. In rats in normal conditions, the slow component seems to be derived chiefly from haemoproteins with different rates of turnover[5,6]. The metabolic source of the initial sharp component remains more enigmatic The rapidity of its formation, only 1–2 h after ^{14}C-glycine administration[3], does not conform to the turnover rate of any known haemoprotein. Here we describe a rapid peak of haem labelling, just preceding the initial bilirubin component, in both liver and young erythroid cells, suggesting that rapidly synthesised haem may be of importance in all the major sites of haem production.

2-^{14}C-glycine (18 mCi mmol^{-1}, New England Nuclear), 40 μCi per 100 g body weight, was given in single intravenous injections to male Sprague–Dawley rats weighing 150–250 g. Animals were fasted for 17 h before the isotope was given. At 0.5, 1, 1.5, 2.5, 4.5, 8, 16, 48 and 96 h, groups of 4–6 rats were exsanguinated by cardiac puncture and red cell haemin isolated for radioassay[3]. Livers were perfused with cold physiological saline, excised, and homogenised in 4 volumes of cold 0.25-M sucrose. A portion of the homogenate was sedimented into mitochondrial and microsomal fractions[7] Bone marrow cells were flushed from both tibias, pressed through stainless steel mesh and washed three times with phosphate-buffered saline. Measured amounts of non-radioactive rat haemoglobin were added to the bone marrow suspensions and the liver fractions, 1% deoxycholate was added as solubiliser, and haemin was crystallised for determination of specific activity[3]. This method yielded 70, 65 and 60% recoveries of ^{14}C-haemin from whole liver, mitochondrial and microsomal fractions respectively, as determined from the radioactivity in livers of animals given ^{14}C-haemin intravenously 5 min before they were killed Reproducibility of ^{14}C-haemin isolation was within 1%.

There were two early peaks of ^{14}C-haem labelling in the liver (Fig. 1) The first was a sharp peak which was maximal 1 h after the administration of ^{14}C-glycine and the second was a more gradual peak maximal at 4.5 h. Qualitatively similar patterns were observed in both the mitochondrial and microsomal fractions, although glycine incorporation into haem was consistently somewhat higher in the latter than in the former. On the basis of assays of NADPH-cytochrome *c* reductase[8] and cytochrome oxidase[7] to determine the recovery of microsomes and mitochondria respectively from the total liver homogenate, it is estimated that the ^{14}C-haem present in both these fractions accounted for 50% of total hepatic haem labelling at 1 h, as compared with 73% at 4.5 h. Studies in rats given ^{51}Cr-labelled red cells indicated that hepatic blood contributed negligibly to ^{14}C-haemin isolated from the liver. There was no histological evidence of extramedullary erythropoiesis in the liver.

Rapid sharp peaks of ^{14}C-haem labelling were also observed in cells from both peripheral blood and bone marrow (Fig. 2) As with the liver, these were maximal 1 h after glycine was given. Both peaks were virtually abolished in rats hypertransfused with volumes of packed red cells equivalent to 3.2% body weight on both the sixth and second days before glycine injection, indicating their origin from immature erythroid cells. The marrow peak was higher than that in peripheral blood. After 1 h marrow haem labelling fell sharply and then increased quickly again, as anticipated for effective haemoglobin synthesis. There was also an abrupt fall in haem labelling in the peripheral blood samples after 1 h. Thereafter, labelling increased gradually over the 96 h of observation, as anticipated from earlier studies[3].

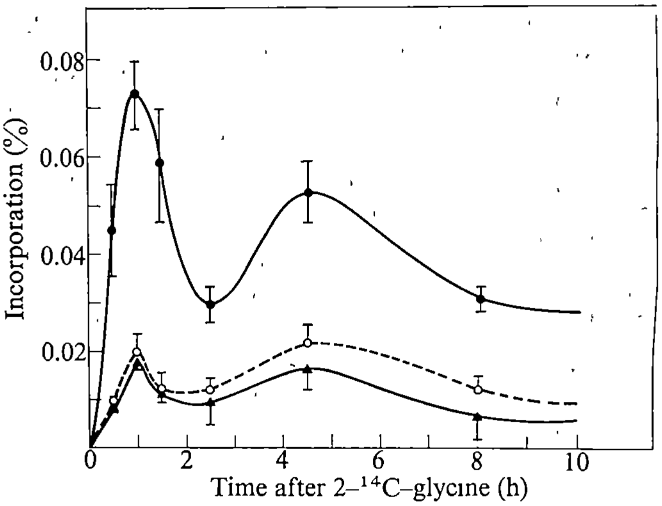

Fig. 1 Incorporation of 2-^{14}C-glycine into liver haem. Each point represents mean ± s.e.m. for 4–6 rats. After 8 h, labelling continued to fall gradually, reaching a stable level between 48 and 96 h. Values for mitochondria (▲) and microsomes (○) are corrected for the efficiency of recovery of these fractions from whole liver (●), based on assays of cytochrome oxidase and NADPH–cytochrome *c* reductase respectively.

A rapid peak of haem labelling in liver has been reported previously[9]. The second component observed in this study was not found, however, perhaps because precursors such as δ-aminolaevulinic acid were used instead of ^{14}C-glycine; the present study was based on the assumption that glycine provides a more physiological representation of haem synthesis[3]. Because of their similar kinetics[3], it is probable that this second phase of hepatic haem labelling is a source of the second phase of early bilirubin production, supporting the conclusion that this bilirubin component arises largely from non-haemoglobin haems in normal rats[3].

Most of the evidence to date has indicated that the initial component of early-labelled bilirubin originates in the liver[9–12]. The demonstration of rapid peaks of haem labelling not only in liver, but also in young erythroid cells, suggests that all these tissues give rise to the rapid 'non-erythroid' fraction of bile pigment. This may explain the hitherto puzzling observation that this bilirubin fraction may be increased in conditions of marked erythroid stimulation[13,14]. In fact, the rapid fall in haem labelling after 1 h in liver, marrow and blood significantly exceeds the formation of labelled bilirubin at this time[3], suggest-

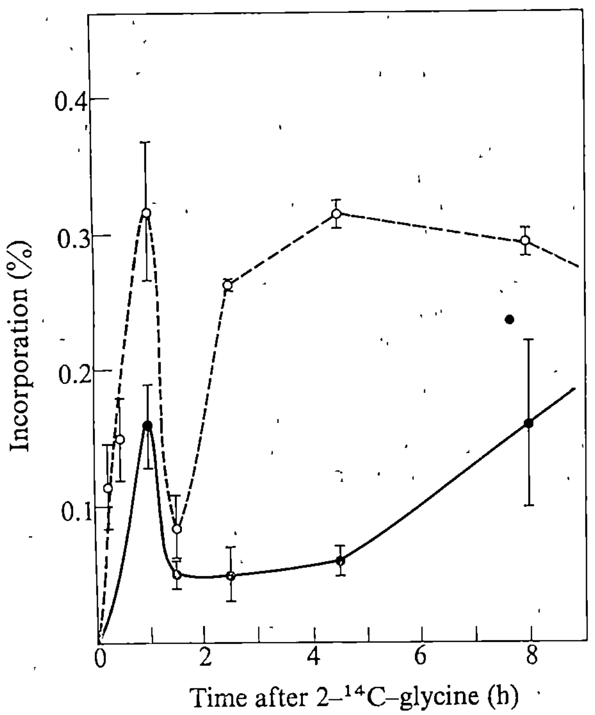

Fig. 2 Incorporation of 2-^{14}C-glycine into haem in peripheral blood and bone marrow cells. Each point represents mean ± s.e.m. for 4–6 rats. After 8 h, haem labelling in marrow cells (○) decreased gradually, reaching a stable level of 0.06% between 48–96 h, while labelling in peripheral blood (●) continued to rise, reaching a value of 0.58% at 96 h. The fraction of the total marrow represented by the assayed tibial cell suspensions was estimated to be 1.3 ± 0.074% as follows: ^{59}Fe in tibial cells collected after maximal plasma ^{59}Fe disappearance at 5 h (4 rats) was compared with maximal ^{59}Fe incorporation into red cells at 4 d (4 rats). This assumes that all ^{59}Fe present in marrow at 5 h would have been recovered in peripheral red cells at 4 d, and probably leads to some underestimation of total marrow mass.

ing that some of the early-labelled haem is degraded to products other than bilirubin[9,15] or that some haem is not catabolised but is altered temporarily to a less extractable form.

The presence of rapid peaks of haem labelling in both of the major sites of haem synthesis—erythroid and hepatic cells—suggests that haem with a rapid rate of turnover is characteristic of all tissues in which haem synthesis takes place. The similar kinetics of the peaks in these different tissues suggest that they all have the same metabolic origin, perhaps a pool of free or unassigned haem, as has been proposed for the liver[5]. There is evidence for some free haem in rabbit reticulocytes[16] and in nucleated erythroid cells from mice[17]. Free haem could serve several functions: to act as a prosthetic group for different haemoproteins, to help govern the rate of overall haem synthesis[18], and to regulate the synthesis of proteins both related and unrelated to haem as a prosthetic group[19].

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Inversion of levels of human T and B cells in early pregnancy

CELL-MEDIATED immunity has been shown to be decreased during pregnancy[1,2]. Finn *et al.*[3] suggested that the cause may be a decrease in the number of T cells; however, levels of T cells were unchanged in women in the third trimester of pregnancy[4]. In addition, the normal number of B cells was found in women who had been pregnant for at least 6 months[5]. Gergely *et al.*[6] also found normal levels of T and B lymphocytes in women between 34 and 36 weeks of gestation. So far no change has been shown in T- and B-cell levels during the latter half of pregnancy.

Here we confirm the earlier results and present evidence for an inversion of the percentage of peripheral blood T and B lymphocytes during early pregnancy. This inversion is evidenced by a relative drop in the percentage of T lymphocytes coupled with an increase in B lymphocytes during the first trimester. These altered T- and B-cell ratios revert to normal during the second trimester and remain normal throughout the rest of pregnancy.

The percentage of T and B cells in Ficoll–Hypaque-purified peripheral blood lymphocytes was determined using direct and indirect rosette techniques[7]. B cells (Ig^+T^-) were identified by rosetting with mixtures of human erythrocytes coated with anti-human κ light chain antibody or anti-λ light chain antibody. T cells (Ig^-T^+) were indirectly rosetted by first sensitising lymphocytes with specific rabbit anti-human T cell antisera. After thoroughly washing these sensitised cells with RPMI 1640 (Grand Island Biological) the T^+ cells were rosetted with human erythrocytes coated with anti-rabbit light chain antibodies.

The percentages of peripheral blood T and B cells were determined for women at different times during pregnancy and compared with average T- and B-cell percentages of four women who were not pregnant (Table 1). From the 7th to 20th week of pregnancy, the percentage of T cells was much lower and the percentage of B cells much higher than in the non-pregnant women (Table 1). On the other hand, from the 22nd week until term, the percentages of B and T cells were essentially the same as in non-pregnant women (Table 1). The change in percentage of B and T cells could result from a unilateral decrease in T cells or an increase in B cells or both. A unilateral change would be reflected by a change in the total lymphocyte count. The number of lymphocytes per mm^3, however, was not significantly different among the patients tested (Table 1), concurring with the observations of Knobloch *et al.*[8]. Thus, the inversion we observed is presumably due to a loss of T cells and simultaneous increase in B cells during the first trimester, followed by a reversion of T and B cells to normal levels during the second trimester. To test this hypothesis, we are doing longitudinal studies of T and B cell levels in pregnant women. The initial blood test of seven women, 11–15 weeks pregnant, have already shown the expected inversion of T- and B-cell percentages in each patient.

Table 1 Percentages of T and B lymphocytes in pregnant and non-pregnant women

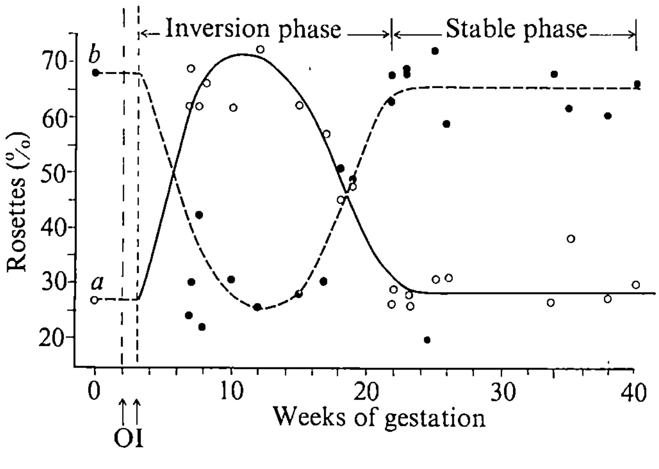
Donor	WBC per mm^3	Lymphocytes per mm^3	Weeks of gestation	% B	% T
Jen	6,400	1,792	NP	28	67
Deb	5,800	1,566	NP	24	71
Any	7,100	1,917	NP	28	70
Mar	6,300	ND	NP	29	64
Bur	7,300	1,606	7	62	30
Hor	4,300	1,247	7	69	24
And	8,200	ND	7.5	62	43
Wil	ND	ND	8	66	22
Bar	5,100	1,326	10	62	31
Hol	ND	ND	12	72	26
Joh	11,200	ND	15	62	28
Flo	7,900	2,607	17	57	31
Wea	6,200	ND	18	45	51
Key	ND	ND	19	48	49
Tho	15,000	ND	22	26	68
Und	ND	ND	22	29	63
Bow	ND	ND	23	26	69
Cam	ND	ND	23	28	68
Smi	5,800	1,160	25	31	72
Bjo	5,300	1,113	26	31	59
Par	5,100	2,244	34	27	68
Bil	ND	ND	35	38	62
Bai	4,000	ND	38	27	60
Can	ND	ND	40 (term)	30	66

NP, non-pregnant; ND, not done; WBC, white blood cells.

When percentages of T and B lymphocytes were plotted against weeks of pregnancy (Fig. 1), our data indicated a biphasic lymphocytic response which separates pregnancy into an early inversion phase and a stable normal phase. The inversion phase occurs during the first half of pregnancy with its peak effect between 7 and 15 weeks of gestation. This phase is characterised by a marked reduction in the percentage of T cells and a concomitant increase in the percentage of B cells. This alteration of normal T and/or B-cell levels during the inversion phase must be related to the implantation of the foetal allograft.

The stable normal phase of the biphasic lymphocytic response occurs during the latter half of pregnancy (week 22 to term) and is characterised by a return to essentially normal T and B cell levels. Since the re-establishment of normal numbers of T and B cells occurs at approximately 22 weeks, the foetal allograft may have already overcome any potential that might have existed for rejection by a T-cell-mediated reaction. Our observations may relate to reports indicating a deficiency in maternal immunological competence during pregnancy. T-cell deficiencies are suggested by the increased incidence of rubella[9] and varicella[10] infections as well as coccidioidomycosis[11] during pregnancy. *In vitro* evidence of altered maternal immunocompetence is based on maternal lymphocyte unresponsiveness to paternal but not unrelated histocompatibility antigens in the mixed lymphocyte reaction[2], and to a loss of maternal T-cell functions as shown by phytohaemagglutin (PHA)-induced transformation[1]. No significant decrease in PHA-induced transformation of maternal lymphocytes *in vitro* was, however, observed by Thiede[12] and Watkins[13]. Other evidence suggests that pregnancy plasma reversibly suppresses the response of maternal and normal lymphocytes in the mixed lymphocyte culture reaction[14] as well as to PHA-induced transformation[15,16]. In view of our results, a re-examination of maternal immunocompetence especially during the T and B-cell inversion phase, seems indicated. In addition, studies with purified T and B cells may be valuable in reassessing the functional immunological capabilities of pregnant women.

Fig. 1 Levels of B (○) and T cells (●) were examined in 20 pregnant women who were in different weeks of gestation. Ovulation (O) was estimated to have occurred by 2 weeks and implantation (I) by week 3. Four women who were not pregnant were tested to determine the average control level of B (*a*) and T cells (*b*).



We suggest that the inversion of T and B-cell levels in early pregnancy may represent a physiological depletion of suppressor T cells (that is cells which are purported to be responsible for the regulation of some B-cell functions[17]) in response to the pregnancy which allows the number of B cells to increase during the first trimester. This increase in B-cell levels may assist in allograft acceptance through the production of blocking antibodies or factors similar to those suggested by the Hellström group for tumours[18] and indeed reported to exist in pregnancy[19,20]. Since the rise and fall of T and B-cell percentages seems to parallel the reported rise and fall of serum human chorionic gonadotrophin (HCG) levels[21], and as Han[22] has reported an inhibitory effect of HCG on the cell mediated immunity of guinea pigs *in vivo* and *in vitro*, it would be intriguing to attribute an immunoregulatory role to this hormone. Women receiving HCG fertility therapy are being tested to examine this possibility.

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Inhibition of mammalian cytotoxic cells by phosphatidylcholine and its analogue

MUCH interest has focused recently on the elucidation of cytotoxic mechanisms in cellular immunology. In addition to complement-mediated cytolysis, two major cell-mediated reactions have been realised from *in vitro* studies: the cytotoxic T-cell[1,2] and K-cell[3,4] systems. The former is characterised by the killing of target cells by cytotoxic T cells taken from donors previously sensitised to target cells, whereas in the latter, target cells are destroyed by normal or unsensitised effectors (K cells), provided IgG antibody to the target cell is present. The exact nature of the K cell is still a matter of controversy[5,6]. Although a voluminous literature exists, little is known about the actual mechanism(s) of action of cytotoxic lymphoid cells. We describe here experiments which implicate phospholipase A activity as an early step of the kill mechanism in K-cell cytotoxicity.

The initial hypothesis for which these experiments were designed is as follows: it can be envisaged that some enzyme on the effector cell surface becomes activated by specific effector cell–target cell contact and alters the molecular architecture of target membrane to result in its lysis. The enzyme may be bound on the effector cell surface or actually be an integral plasma membrane protein. Such a mechanism could explain how the effector cell escapes being killed in the process, for it does remain functional, in both the K-cell[7] and T-cell[5] systems. One likely candidate enzyme would be phospholipase A (EC 3.1.1.4), for its activity leads to the conversion of lecithin (phosphatidylcholine) to lysolecithin+fatty acid, the former product being a very potent lytic agent. Using the rationale that such enzyme activity would be inhibited by various agents which should inhibit this activity, a synthetic inhibitor of phospholipase A (Rosenthal's inhibitor) and a normal phospholipid substrate for this enzyme were tested for possible inhibitory effects in a murine K-cell cytotoxic system.

The assay system used is a modification from Perlmann and Perlmann[7], consisting of spleen effector cells from normal BALB/c female mice+adult chicken erythrocytes (^{51}Cr-labelled)+BALB/c hyperimmune antiserum against adult chicken erythrocytes.

Rosenthal's inhibitor[8], or DL-2,3-distearoyloxypropyl-(dimethyl)-(2-hydroxyethyl)-ammonium acetate (Calbiochem), a synthetic analogue of lecithin and inhibitor of phospholipase A, was examined for inhibitory capacity in this assay system. Figure 1 shows the dose–response effect of this analogue on the cytotoxic reaction; a 50% inhibition is noted at 6×10^{-5}M. At an inhibitor concentration that results in more than 90% inhibition, no greater than a 10% reduction in spleen cell viability was noted, as judged by Trypan blue exclusion. Thus the inhibition effect cannot be due to simple nonspecific toxicity. Interference with the

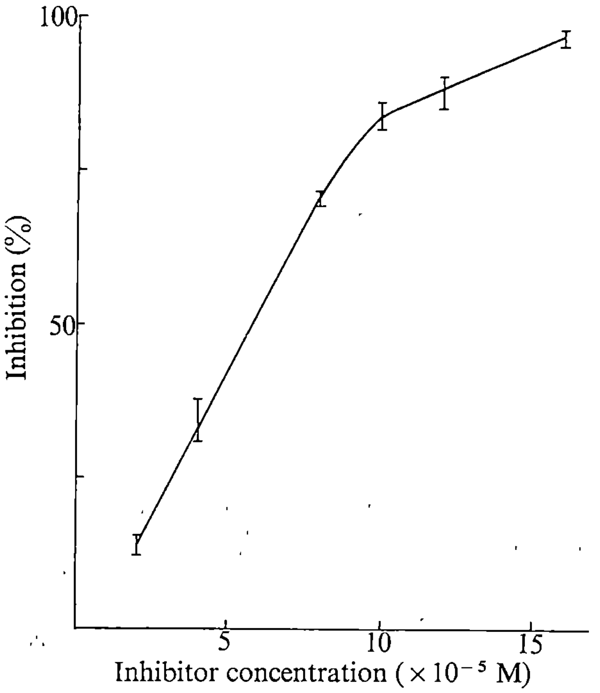

Fig. 1 Dose–response relationship of Rosenthal's inhibitor in murine K-cell cytotoxicity. Cytotoxic cultures (in triplicate) consisted of 2.5×10^6 BALB/c spleen cells $+10^5$ chicken erythrocytes, ^{51}Cr-labelled, ± BALB/c anti-chicken erythrocyte antiserum (1/1,500 final concentration) in a total volume of 1.5 ml medium 199 containing FCS (5%), penicillin (100 U ml^{-1}) and streptomycin (100 μg ml^{-1}). Incubation was performed in a 10% CO_2, high humidity incubator at 37 °C. Rosenthal's inhibitor was added at various concentrations, and the cultures were incubated overnight (19 h). Results are expressed in terms of percentage inhibition of cytotoxicity:

$$1-\frac{\%\text{ specific release in experimental}}{\%\text{ specific release in control}}\times100$$

The percentage specific release is defined as the percentage release in cultures containing anti-target cell antiserum minus the percentage release in cultures containing normal mouse serum.

Table 1 Reversibility of PC inhibition

Experiment	Prewash conditions	% Specific release at 1.5 h	3.0 h	4.0 h	4.5 h
1	Hanks'	5.8 (0.5)*	26.5 (0.9)	31.3 (2.2)	—
	PC 100	0.3 (0.7)	21.5 (0.5)	27.1 (0.5)	—
2	Hanks'	7.3 (1.3)	21.9 (0.5)	26.2 (2.3)	—
	PC 100	3.5 (0.8)	16.5 (1.4)	20.9 (2.0)	—
3†	Hanks'	8.1 (0.7)	29.5 (1.8)	—	38.3 (3.6)
	PC 100	3.7 (0.5)	23.7 (0.7)	—	33.4 (0.8)
4	Hanks'	6.1 (0.5)	28.1 (1.3)	33.1 (1.8)	—
	PC 200	1.3 (0.4)	9.8 (0.6)	13.6 (0.4)	—
5	Hanks'	5.4 (0.6)	37.2 (1.9)	—	42.3 (0.6)
	PC 200	2.1 (0.8)	13.5 (1.5)	—	17.4 (1.5)
6†	Hanks'	5.3 (0.7)	37.4 (0.1)	48.2 (0.4)	—
	PC 200	2.1 (0.4)	30.6 (1.5)	41.6 (0.1)	—
7†	Hanks'	12.1 (0.6)	55.8 (0.9)	65.6 (0.7)	—
	PC 200	4.1 (0.4)	50.1 (0.1)	60.8 (3.0)	—

Cytotoxic mass cultures, in the presence or absence of PC at 100 (PC 100) or 200 (PC 200) μg ml^{-1} final concentration, were incubated at 37 °C for 90 min; aliquots were then removed to determine percentage specific release and the remaining cells were washed free of PC by centrifugation through a 20% (w/v) bovine serum albumin solution prepared in Hanks' BSS. The washed cells were resuspended in fresh medium and reincubated for further 90, 150 or 180 min to assess the degree of reversibility.

*Standard deviation.

†Experiment done in presence of 5% FCS.

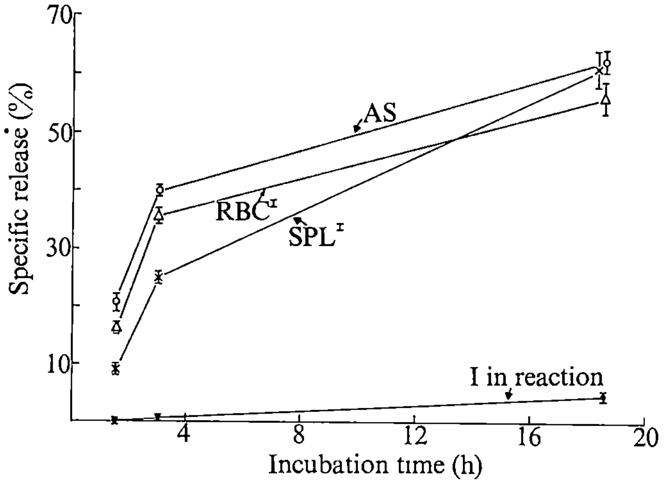

Fig. 2 Rosenthal's inhibitor pretreatment of effector or antibody-coated target cells. Spleen effectors or antibody-coated targets were pretreated with Rosenthal's inhibitor (8×10^{-6} M) for 30 min at 37 °C, washed in medium 199 and tested for cytotoxicity after various times. ○, Positive control, no inhibitor; △, antibody-coated target cells pretreated with inhibitor; ×, spleen effector cells pretreated with inhibitor; ●, inhibitor present during cytotoxic reaction.

recognition phase of the reaction (K cell Fc-receptor attachment to the Fc portion of target-bound antibody) is unlikely as EA rosette formation (mouse spleen + rabbit anti-SRBC-coated SRBCs (sheep red blood cells)) is unaffected by Rosenthal's inhibitor. As one would expect of a competitive inhibitor, pretreatment of effector or target cells was not very effective for determining the cell type affected (Fig. 2); however, the reduced cytotoxicity at early times for the pretreated spleen cells (56% at 1.5 h; 37% at 3.0 h) suggests that the inhibitor affects predominantly the effector cell population. Reduction is not attributable to diminished numbers of effector cells, for both control and pretreated spleen cells were adjusted to the same number of viable cells.

Phosphatidylcholine (PC) was next examined for possible inhibition of the cytotoxic reaction. Addition of distearoyl-PC (Calbiochem) to the cytotoxic cultures caused inhibition —50% at 37 μg ml^{-1} or 4.6×10^{-5}M (Fig. 3). As for Rosenthal's inhibitor, PC had no effect on EA rosette formation. The inhibition by PC at 100 μg ml^{-1} was completely reversible, with no time lag, following removal of the phospholipid by centrifugation and resuspension in fresh medium; at 200 μg ml^{-1}, however, the reversal was complete only in the presence of serum in the cultures (Table 1) while in the other experiments, presence or absence of serum had no effect. The site of PC inhibition seems to be on the effector cell, for spleen cell pretreatment with PC results in diminished effector cell function, provided pretreatment is carried out in the absence of foetal calf serum.

The protective effects of Rosenthal's inhibitor and PC against target cell injury strongly suggest that phospholipid alteration by phospholipase A is the basis for the loss of membrane integrity in these cytotoxic reactions. The fact that neither agent affects EA rosetting efficiency argues against interference with cell–cell contact as the mechanism whereby these substances inhibit the cytotoxic reaction. The most probable manner by which PC inhibits the cytotoxic reaction is as follows: exogenous PC may act as a substrate for phospholipase A, thus preventing the enzyme from attacking endogenous target cell membrane phospholipids. The lysophosphatidylcholine (LPC) thus generated by the enzyme activity would not subsequently lyse the target cell since excess PC and effector cell surface would be expected to bind preferentially the LPC generated, because of their more intimate association with the proposed enzyme sites on the effector cell. The fact that reversibility of PC inhibition at 200 μg ml^{-1} is only partial in the absence of serum strongly indicates that LPC is generated and interferes with effector cell function. Effector integrity is not affected when serum is present in the reaction mixture, and the reason for the protective effect of serum probably lies in the fact that serum albumin binds LPC and renders it non-lytic[9].

It is interesting that the observed PC inhibition parallels that of EDTA[10] in that both seem to act at a late stage in the cytolytic reaction (since there is no time lag in chromium release when inhibitor is removed). As most phospholipase A enzymes are calcium dependent[11] and thus inhibited by EDTA, it is tempting to speculate that both PC and EDTA act at the same point in the cytotoxic sequence, namely at that point requiring phospholipase A activity.

Experiments are in progress to assess the feasibility of obtaining direct chemical evidence for the generation of LPC in the target cell membranes during the cytotoxic reaction.

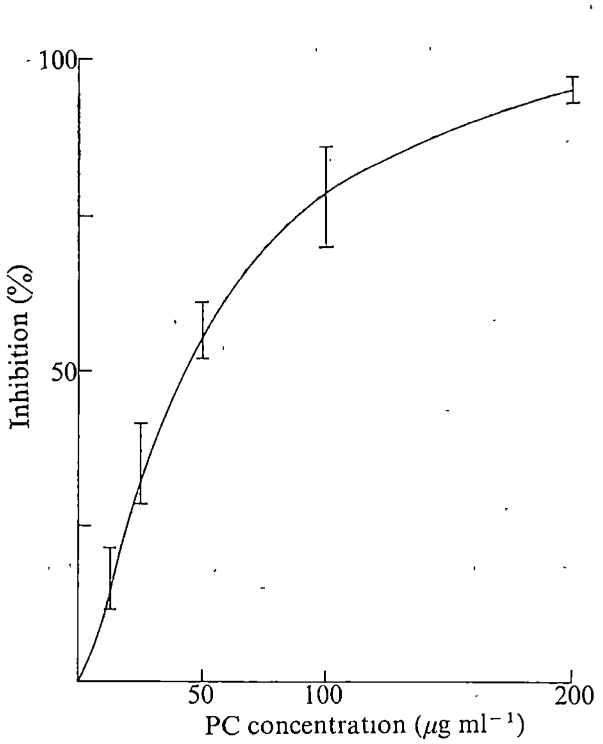

Fig. 3 PC inhibition of murine K-cell cytotoxicity. Same conditions as in Fig. 1, except that incubation was for 3 h and reactions were carried out in the absence of foetal calf serum. To obtain an aqueous dispersion, PC was sonicated in Hanks' BSS for 5 min, using a Biosonik IV sonicator (low setting 50). The results are expressed as the means ± s.d. of three experiments.

Any hypothesis implicating phospholipase A in the kill phase mechanism must not overlook the fact that most such enzymes have been found to be incapable of attacking the phospholipids of intact plasma membranes[12]; only in the presence of membrane-perturbing agents such as hypotonic milieu[13], detergents[14] or DLF[15] (direct lytic factor, a small basic peptide found in snake venoms) do such enzymes catalyse the conversion of intact membrane phospholipids into their lyso-derivatives. Consequently, a two-step reaction can be postulated: a preparatory step in which the membrane is perturbed, and a second step in which phospholipase A is active. Efforts are under way to assess the possible nature of such a preparatory step.

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Immunological restoration of both thymectomised and athymic nude mice by a thymus factor

AN endocrine function for the thymus has been suggested by experiments using both thymic tissue enclosed in diffusion chambers and cell-free thymus extracts to restore the immunological functions in neonatally thymectomised animals[1–4]. Several investigators have prepared a variety of thymic humoral factors (thymic hormones) capable of restoring different immunological functions[5], but so far they have had little success in endowing congenitally athymic nude mice with T cells and T-dependent functions[6], although thymus grafts work very well[7]. This has led to the suggestion that thymic humoral factors may work by promoting the maturation of lymphocytes which have already passed through the thymus. The study presented here, however, shows evidence that a thymus factor substitutes for the thymus.

The first step was to verify *in vivo* the capacity of a thymus factor (TF) to reject tumour allografts in thymectomised and athymic nude mice. The assay method of purification of thymic hormone has presented many inherent difficulties especially *in vitro:* for example, it has been reported that a great variety of mitogenic and non-mitogenic substances, including those which raise the intracellular concentration of cyclic AMP, can induce *in vitro* the differentiation of stem cells to θ-positive lymphocytes and immunocompetent cells[8–10].

The TF was prepared from calf thymus using a modification of the method described by Goldstein *et al.*[11]. The same procedure, up to and including the heat treatment, was followed. Then saturated $(NH_4)_2SO_4$ (pH 6.8) was added to a concentration of 50% saturation. The precipitate was collected by centrifugation and dissolved in saline. Free $(NH_4)_2SO_4$ was removed from this solution on a column of Sephadex G-25. The protein fractions were combined and diluted with saline to a final protein concentration of either 1 or 2 mg ml^{-1}. The same fractionation procedure from calf spleen was performed as a control.

Fibrosarcoma cells, induced originally by a subcutaneous injection of 3-methylcholanthrene into a C3H/He (H-2^k) mouse, were used as tumour grafts. A/J (H-2^a) mice were used as recipients of tumour allografts in standard laboratory conditions. In the first experiment, A/J mice were thymectomised within 24 h after birth. From the following day, the mice were subcutaneously injected five times a week with 0.2 mg of either TF, or, as controls, spleen extract (SE) or bovine serum albumin (BSA). On the day after the 10th injection, 2×10^6 fibrosarcoma cells were inoculated subcutaneously into the backs of the mice. After a further 20 injections with TF, SE, or BSA, the tumour incidence was noted in each group. In the 24 mice injected with TF, no tumours were found, whereas, of the 20 mice injected with BSA, 14 developed tumours and the remaining 6 died of 'wasting disease' before tumour development Of the 22 mice injected with SE, 13 developed tumours and the remaining 9 died of 'wasting disease' In the second experiment, A/J mice were thymectomised 3–5 d after birth to avoid death from wasting disease following neonatal thymectomy. The experimental schedule followed after the thymectomy is shown in Table 1. Of the 34 mice injected with TF, 31 rejected the tumours and survived the 90-d period. Of the mice injected with BSA or SE no tumours were rejected, and after the 90-d period there were none surviving (Table 1). In the third experiment, athymic nude mice (backcrossed on to BALB/c (H-2^d) mice) were used Nude mice obtained from the Central Institute for Experimental Animals, Tokyo, were raised in specific pathogen-free conditions. Male athymic nude mice (4 weeks old) were subcutaneously injected every day with 0.5 mg of either TF or SE. In the case of athymic nude mice, no rejection took place when TF was injected after inoculation of the tumour cells. No tumours were, however, detected when 2×10^6 fibrosarcoma cells were inoculated into 5 athymic nude mice which had been pretreated 60 times with TF. Histological findings showed a remarkable accumulation of lymphocytes in the location of the tumours undergoing rejection. Moreover, to verify *in vitro* the cytotoxic effects on the tumour cells, a microcytotoxicity assay was used[12]. Spleen cells from both thymectomised and athymic nude mice which had rejected tumours showed evidence of contact with the tumour cells followed by destruction of the latter. Nonetheless, incubation with 1 : 4 diluion (final dilution 1 : 12) of the anti-Thy 1.2 (θ-C3H) serum and complement completely abolished the cytotoxic activity of their spleen cells (Table 2). In the treatment of

Table 1 The effect of injection of a thymus factor into thymectomised A/J mice on the rejection of allogeneic tumour grafts of greatest diameter of between 5 and 10 mm

Injection		No. of mice rejecting tumours	No. of mice dead within 30 d	No. of mice surviving 90 d
Bovine serum albumine	(BSA)	0/24 (0%)	8/24 (33%)	0/24 (0%)
Thymus factor	(TF)	31/34 (91%)	3/34 (9%)*	31/34 (91%)
Spleen entract	(SE)	0/24 (0%)	6/24 (25%)	0/24 (0%)

A/J (H-2^d) mice were thymectomised 3–5 d after birth. Two days later, 2×10^6 fibrosarcoma cells of C3H/He(H-2^k) origin were inoculated subcutaneously into the backs of the mice. After a period of 10–20 d, when the tumour size reached 5–10 mm at the greatest diameter, the mice were subcutaneosuly injected five times a week with 0.5 mg of BSA, TF, or SE. After 20 injections, the number of mice rejecting tumours was noted.

*Statistically significant difference from the control (BSA) values, $P < 0.05$.

Table 2 *In vitro* cytotoxic effects of spleen cells and sera from thymectomised A/J mice and athymic nude mice subjected to long term injections of either thymus factor (TF) or spleen extract (SE), on allogeneic tumour cells

			% Cytotoxicity (mean±s.e.m.)				
			Cell-mediated cytotoxicity* (at 48 h)			Antibody-mediated cytotoxicity (at 12 h)	
Mouse†	Injection	Tumour rejection		Treatment with anti-θ serum + C			
				Anti-θ serum dilution		Mouse serum dilution	
				1:4	1:16	1:1	1:4
Tx A/J (5)	SE (0.5 mg×20)	no	6.3±2.2	1.2±0.5	ND	8.5±3.2	ND
Tx A/J (5)	TF (0.5 mg×20)	yes	89.4±8.3	1.8±0.3	22.3±6.1	19.5±4.8	8.6±3.2
nu/nu (3)	SE (0.5 mg×60)	no	1.8±0.4	ND‡	ND	1.3±0.4	ND
nu/nu (3)	TF (0.5 mg×60)	yes	62.5±6.3	2.1±0.6	24.7±5.3	16.5±6.8	9.0±4.1

A microcytotoxicity assay was performed by using spleen cells and sera from both thymectomised and athymic nude mice which had rejected tumour allografts following TF-treatment, and also from those which had shown no tumour rejection.

*Effector–target cell ratio = 50:1.

†Numbers in brackets indicate the number of mice in the group.

‡ND, not done.

their spleen cells with complement alone, anti-Thy 1.2 serum alone, and normal AKR mouse serum plus complement, none of them abrogated the cytotoxic activity of their spleen cells. In comparison with their spleen cells, sera from both thymectomised and athymic nude mice which had rejected tumour allografts following TF-treatment showed only a slight cytotoxicity. In the experiments, cell-mediated cytotoxicity had a far more important role than either cell-mediated cytotoxicity which was antibody-dependent (data not shown) or antibody-mediated cytotoxicity. The results show that a thymus factor induces, in both thymectomised and athymic nude mice, 'killer T cells' which have a major role in the rejection of tumour allografts.

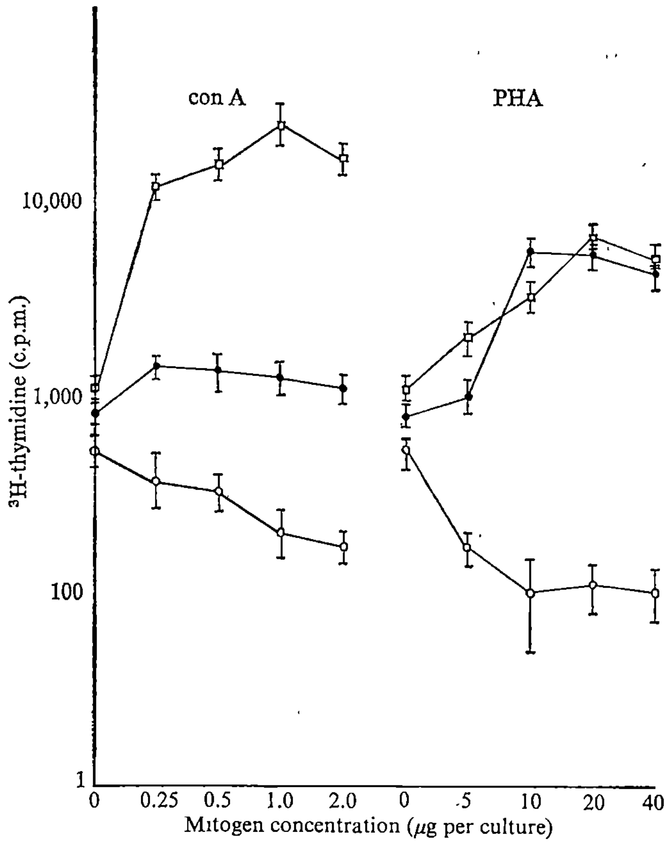

Fig. 1 Effect of a thymus factor (TF) on the responsiveness to PHA and con A of *nu/nu* spleen cells Two four-week-old male *nu/nu* mice were subcutaneously injected every day with 0.5 mg of TF for 60 d. Three days later, the mitogenic reactivity of their pooled spleen cells was determined by measuring the incorporation of ^{3}H-thymidine into DNA. ○, Untreated *nu/nu* mice; ●, TF treated *nu/nu* mice; □, BALB/c mice. Vertical bars and points show the mean±s e.m.

The next step was to consider whether helper T cells could be induced by TF in athymic nude mice. Nude mice which had been injected 60 times with 0.5 mg of TF or SE were used. On the third day after the last injection, the mice thus treated, and untreated *nu*/+ control mice, were immunised intraperitoneally with 4×10^8 sheep red blood cells (SRBCs). Five days later the number of haemolytic plaque-forming cells (PFCs) in their spleens was determined using Cunningham's[13] technique. The direct splenic PFC response of the TF-treated *nu/nu* mice was increased to 9,526±2,326 and the indirect PFC response to 92,160 ±4,366, both of which were near the normal level of *nu*/+mice. The response of the SE-treated *nu/nu* mice, on the other hand, did not show a statistically significant difference from that of the untreated *nu/nu* mice (Table 3)

Table 3 The effect of a thymus factor on the number of direct and indirect PFCs in the spleens of *nu/nu* mice

Mouse	Injection	PFCs per spleen after 5 d*	
		Direct	Indirect
nu/nu	No	176 ± 152	1,123 ± 221
nu/nu	TF (0.5 mg×60)	9,526 ± 2,326	92,160 ± 4,366
nu/nu	SE (0.5 mg×60)	246 ± 110	1,236 ± 312
nu/+	No	19,932 ± 8,421	108,415 ± 21,513

Four-week-old male *nu/nu* mice were subcutaneously injected every day with 0.5 mg of TF or SE for 60 d. On the third day after the last injection, all three mice in each of the four groups were immunised intraperitoneally with 4×10^8 SRBCs. After 5 d, the number of PFCs in the spleens was determined

* Means ± s.e m.

Histological findings in the lymph nodes and spleens from the TF-treated *nu/nu* mice showed an accumulation of lymphocytes in T-cell dependent areas. Moreover, to verify T-cell induction in the TF-treated *nu/nu* mice, the cytotoxicity test using anti-Thy 1.2 serum and complement was performed as described by Boyse *et al*[14]. Spleen cells and lymph node cells from the SE-treated *nu/nu* mice showed cytotoxic indices of 0.5 and 0.7%, respectively, whereas spleen cells and lymph node cells from the TF-treated *nu/nu* mice showed indices of 23 and 39%, respectively. These findings confirm the induction of T cells by TF in *nu/nu* mice.

The next experiment was to determine the capacity of TF to restore lymphocyte responsiveness to the T-cell mitogens phytohaemagglutinin (PHA) and concanavalin A (con A) in TF-treated *nu/nu* mice. We used pooled spleen cells from two *nu/nu* mice which had received the same treatment with TF as in the experiment already described. Age-matched and sex-matched controls consisted of untreated *nu/nu* and ordinal BALB/c mice. The mitogenic reactivity of each spleen cell was determined by measuring the incorporation of ^{3}H-thymidine into DNA. We then set up 5×10^5 spleen cells in 0.2 ml of culture medium (RPMI 1640 medium containing 10% foetal calf serum) in wells of microtrays (Limbro) and cultured them in triplicate for 72 h. We added 10 μl of culture medium containing the concentrations of PHA-P (Difco) and con A (Calbiochem.) indicated in Fig. 1. Twenty-four hours before collection, 1 μCi of ^{3}H-thymidine was introduced into each well. Labelled material was measured using liquid scintillation counting. Spleen cells from the untreated *nu/nu* mice did not respond to either of the mitogens (Fig. 1). In contrast, spleen cells from the *nu/nu* mice treated with TF showed a remarkable response to PHA. The level was almost the same as that shown by the BALB/c mice. In contrast with the response to PHA, the *nu/nu* spleen cells treated with TF did not show a response to con A of a level comparable with that of the BALB/c mice, although they did show a statistically significant difference from that of the untreated *nu/nu* spleen cells ($P<0.001$). It is difficult to explain this dissociation of mitogenic responses to PHA and con A, but T cells induced by this thymus factor may correspond to the TH-2b subclass described by Dyminski *et al.*[15], which responds significantly to PHA and SRBC but poorly to con A and mixed lymphocyte culture (MLC). Van Bekkum *et al.*[7] have reported that it is impossible to restore the PHA responsiveness of athymic nude spleen cells after long term treatment *in vivo* with thymus extracts (thymosin and THF). Our study, however, has demonstrated that the thymus factor is capable of restoring PHA responsiveness and the other T-dependent functions in athymic nude mice. This thymus factor is still crude and may turn out to be a mixture of a few active components of the thymus; it is also possible that more than one component of thymus factor are needed for thymic hormone to substitute for the thymus.

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Calcium-dependent stimulation of platelet aggregation by PGE_2

THERE is considerable interest in the effects of prostaglandins on blood platelets, partly because the synthesis of prostaglandins by stimulated platelets is important in haemostasis, and partly because platelets can be used as models to investigate the actions of prostaglandins (PGs) at a cellular level[1]. The effects of different PGs on platelets are diverse: PGE_1 is a potent inhibitor of platelet aggregation[2] with a K_i value around 30 nM and PGD_2 is an even more effective inhibitor of aggregation of human platelets, but not those of some other species[3,4]. PGE_2 inhibits platelet aggregation at high concentrations ($> 1 \times 10^{-6}$ M), and there is some controversy as to whether lower concentrations enhance aggregation[5]. When human platelets are stimulated by collagen they synthesise PGE_2 from arachidonic acid[6] and the cyclic endoperoxide intermediates in the biosynthesis of PGE_2 exert a direct aggregating effect[7,8]. Synthetic derivatives of PGE_2 can also induce the aggregation of human platelets[9], but PGE_2 itself has never been found to induce aggregation directly.

Previous studies of the effects of PGs on platelet aggregation used platelets suspended in citrated plasma (with most of the calcium chelated) or in calcium-free artificial media. The effects of PGE_1 on several biological systems are influenced by the ionised calcium concentration[10,11] and platelet aggregation is also calcium dependent to a variable extent in different animal species[12]. We have investigated the effects of PGE_1, PGE_2 and PGD_2 on platelet aggregation in heparinised and citrated platelet-rich plasma (PRP) from man, pig and rat, and have found that PGE_2 reproducibly enhances aggregation in heparin PRP (but not in citrate PRP) from man and rat, and induces aggregation directly in pig heparin PRP.

Table 1 Inhibition of collagen-induced platelet aggregation by prostaglandins

Species	Anticoagulant	Concentration ($\times 10^{-6}$ M) inhibiting aggregation by 50%		
		PGE_1	PGD_2	PGE_2
Man	Citrate	0.015	0.008	6.0
	Heparin	0.054	0.015	22.0
Rat	Citrate	0.06	>200	75
	Heparin	0.09	>200	135
Pig	Citrate	0.27	1.4	67
	Heparin	0.12	0.15	*

*Directly induces aggregation

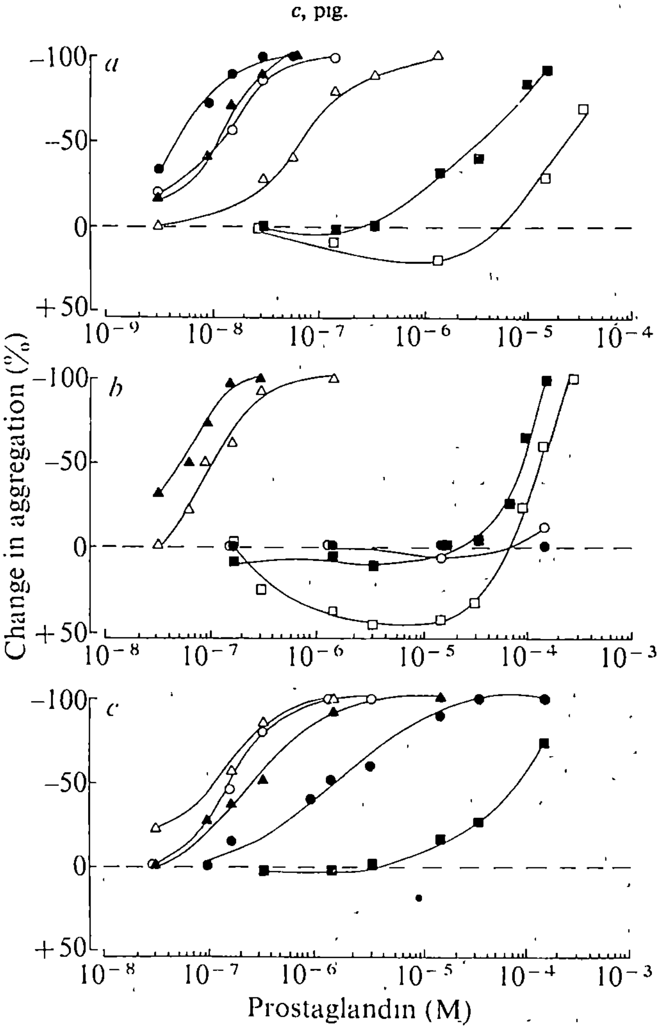
Prostaglandins or saline were preincubated for 1 min at 37 °C in PRP before the addition of collagen. In each experiment, the dose of collagen producing a half-maximal rate of aggregation was used as the aggregation stimulus. Results are expressed as mean IC_{50} values ($\times 10^{-6}$ M) of three determinations.

Human blood was obtained by antecubital venepuncture from volunteers who had ingested no aspirin within the previous 10 d. Pig blood was obtained from a cannula inserted into the carotid artery of animals under Nembutal anaesthesia, and rat blood was obtained by venepuncture from the inferior vena cava of animals under light ether anaesthesia. The preparation of PRP and the measurement of platelet aggregation induced by collagen have been described in detail[13,14]. Drugs or saline were preincubated in PRP for 1 min at 37 °C before the addition of collagen. To compensate for minor variations in the platelet aggregation response during the course of the experiments, controls were performed before and after each test determination. PGE_1, PGE_2 and PGD_2 were dissolved in 95% ethanol and converted to the sodium salt by addition of 9 volumes of sodium carbonate (1.9×10^{-3} M). Stocksolutions (3×10^{-3} M) were stored under nitrogen at −20 °C. Fresh samples were thawed before each experiment, and diluted with iso-osmotic saline.

The potencies of PGE$_1$, PGE$_2$ and PGD$_2$ as inhibitors of platelet aggregation in the three species tested are shown in Table 1. PGE$_1$ was the only PG which inhibited aggregation in both heparin PRP and citrate PRP in all three species. It was most potent in man, slightly less so in the rat, and least potent in the pig. The IC$_{50}$ values (concentration producing 50% inhibition of aggregation) ranged from 0.015×10^{-6} M in human citrate PRP to 0.27×10^{-6} M in pig citrate PRP. PGD$_2$ was more potent than PGE$_1$ in man (IC$_{50}$ = 0.008×10^{-6} M in citrate PRP), less potent than PGE$_1$ in the pig, and was inactive in the rat. PGE$_2$ was much less potent than PGE$_1$ in all species, with IC$_{50}$ values in citrate PRP of 6×10^{-6} M in man, 67×10^{-6} M in the pig, and 75×10^{-6} M in the rat. In man and rat, all three PGs were more potent inhibitors in citrate PRP than in heparin PRP. In the pig, the reverse was true, and PGE$_2$ was unique in causing aggregation directly in pig heparin PRP. PGE$_2$ never induced aggregation in human and rat PRP, but with concentrations in the range 0.3×10^{-6}–30×10^{-6} M in the rat and 0.3×10^{-6}–3.0×10^{-6} M in man, collagen-induced aggregation was reproducibly enhanced in heparin PRP though not in citrate PRP. Dose–response curves for the enhancement and inhibition of aggregation by PGE$_2$, and for inhibition of aggregation by PGD$_2$ and PGE$_1$ are shown in Fig. 1.

When PGE$_2$ was added to pig heparin PRP at concentrations $\geq 0.5\times10^{-6}$ M there was an immediate aggregation response which was not preceded by any detectable change in platelet shape. The aggregation response was dose dependent, being small and reversible at concentrations $\sim0.5\times10^{-6}$–5.0×10^{-6} M, and larger and more slowly reversible at higher concentrations (Fig. 2*a*). Aggregation induced by PGE$_2$ in pig heparin PRP was inhibited by EDTA (3×10^{-3} M), trisodium citrate (12.5×10^{-3} M), PGE$_1$ and PGD$_2$ (Fig. 2*b*); the aggregating effect of a given concentration of PGE$_2$ could be abolished by a concentration of PGE$_1$ 100 times lower. Platelet aggregation induced by PGE$_2$ in pig heparin PRP did not depend on the presence of heparin; similar aggregation responses were obtained when PGE$_2$ was added to partially recalcified pig citrate PRP.

Fig. 1 Dose–response curves for the modification of collagen-induced aggregation by PGE$_1$ (▲, △), PGE$_2$ (■, □) and PGD$_2$ (●, ○) in citrate PRP (▲, ■, ●) and heparin PRP (△, □, ○) of man, pig and rat. The aggregation stimulus in each case was the concentration of collagen producing a half-maximal rate of aggregation. Results (mean values, *n* = 3) are expressed as percentage change in the rate of aggregation. *a* Human; *b*, rat; *c*, pig.



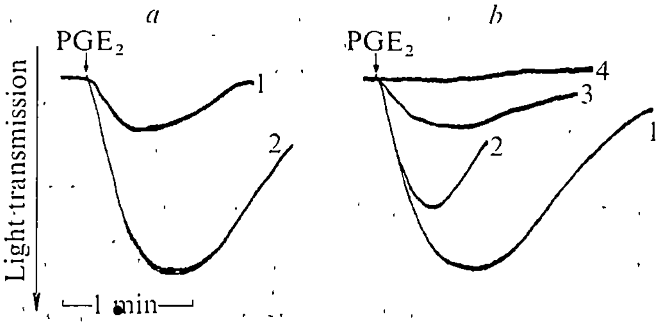


Fig. 2 *a*, Platelet aggregation responses induced by PGE$_2$ in pig heparin PRP. 1, 1.5×10^{-6} M PGE$_2$; 2, 1.5×10^{-5} M PGE$_2$. *b*, Inhibition of aggregation induced by PGE$_2$ in pig heparin PRP. PGE$_2$ (1.5×10^{-5} M) was added to samples preincubated for 1 min with: 1, saline; 2, EDTA (3×10^{-3} M); 3, PGD$_2$ (1.5×10^{-6} M); 4, PGE$_1$ (1.5×10^{-7} M).

Vigdahl *et al.*[10] showed that ionised calcium antagonised the inhibitory effect of PGE$_1$ on ADP-induced aggregation of human platelets. Our results suggest that this antagonism is not limited to PGE$_1$, nor to platelet aggregation induced by ADP, since inhibition of collagen-induced aggregation of human and rat platelets by PGE$_1$, PGE$_2$ and PGD$_2$ was less in heparin PRP than in citrate PRP. Pig platelets were unusual in that PGE$_1$ and PGD$_2$ were more potent inhibitors in heparin PRP than in citrate PRP. The reason for this discrepancy is unknown, but several observations have demonstrated that the function of calcium in pig platelet aggregation is different from that in other species[12,15,16]. Indeed, the modification of PG effects by calcium is most marked in the case of PGE$_2$ in pig platelets: PGE$_2$ inhibited aggregation in citrate PRP, but induced aggregation directly in heparin PRP or recalcified citrate PRP. This aggregation induced by PGE$_2$ is the first demonstration of a direct platelet-aggregating effect induced by a stable, naturally-occurring prostaglandin. PGE$_2$ has been shown to potentiate platelet aggregation induced by endoperoxide intermediates in the PGE$_2$ biosynthetic pathway, and such aggregation (measured in human citrate PRP) was also enhanced by the addition of ionised calcium[17]. Our observation that collagen-induced aggregation of human and rat platelets in heparin PRP can be enhanced by subinhibitory concentrations of PGE$_2$ supports the concept that PGE$_2$ can exert a stimulatory effect on platelets through a calcium-dependent mechanism. It seems likely that the stimulatory effect of PGE$_2$ on human and rat platelets, and its direct aggregating effect on pig platelets, could be mediated by an influx of extracellular calcium; platelets can be stimulated in this way by the calcium ionophore A23187 (ref. 18) and PGE$_1$ can stimulate calcium influx into other tisues [11,19]. It is generally accepted that inhibition of platelet aggregation by PGs is mediated by stimulation of adenylate cyclase[5,20], but 10^{-5} M PGE$_2$ is required to stimulate human platelet adenylate cyclase, and collagen-induced aggregation of human platelets in heparin PRP is stimulated by PGE$_2$ at concentrations below 10^{-6} M. Similarly, the minimal effective concentration of PGE$_2$ which induces aggregation in pig heparin PRP is about 10^{-6} M, and the minimal inhibitory concentration in pig citrate PRP is around 10^{-5} M. Further studies are necessary to determine whether PGE$_2$ can, in fact, stimulate the influx of calcium into platelets at concentrations lower than those which stimulate adenylate cyclase. Investigations of the mechanisms by which PGs exert their various biological effects are facilitated by the use of a homogeneous population of cells on which responses to different PGs can be readily determined. Since pig platelets contain receptors for PGE$_1$, PGE$_2$ and PGD$_2$, and both stimulatory and inhibitory effects can be obtained, the pig platelet could prove a valuable model for studying the interactions of calcium, prostaglandins and their cellular receptors.

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Human breast cancer responsive to androgen in long term tissue culture

Although a significant role has been established for oestrogen in the growth of some animal[1,2] and human[3,4] mammary carcinoma, evidence for androgen dependency of human breast cancer has remained equivocal. Although the existence of a mouse mammary carcinoma which shows modest androgen responsiveness[5] has supported the notion that some human breast cancer might also be dependent on androgens, no direct proof has been available. We have described our work characterising the oestrogen responsiveness of MCF-7, a human breast cancer cell line maintained in tissue culture for over 3 yr (ref. 6). This cell line was shown to contain oestrogen receptor, to be killed by antioestrogens, and stimulated by physiological concentrations of oestradiol. We now describe further investigations of this cell line which reveal that in addition to oestrogen responsiveness, this cell line shows threefold enhancement of precursor incorporation into macromolecules by androgens using serum-free conditions which preclude stimulatory effects of other trophic hormones. Furthermore, this stimulation by androgens seems to be mediated by interaction with a cytoplasmic androgen receptor protein which is clearly differentiable from the oestrogen receptor also found in these cells. Aside from defining an interesting new system in which the action of androgen can be studied in a human cell line in tissue culture, the present study provides unequivocal evidence for the androgen responsiveness of some human breast cancer at least *in vitro*.

Table 1 shows the effects of various steroids on incorporation of radiolabelled thymidine and leucine into acid precipitable material. As indicated, 5-α-dihydrotestosterone stimulates leucine and thymidine incorporation three- to fourfold above control levels. Testosterone also stimulates both thymidine and leucine incorporation though to a lesser degree than 5-α-dihydrotestosterone. At similar concentrations 5-β-dihydrotestosterone was inactive in stimulating macromolecular synthesis. R2956 (17-β-hydroxy-2,2,17-α-trimethyl-estra-4,9,11-trien-3-one), an antiandrogen provided by Dr J. P. Raynaud (Roussel, France)[7], at a concentration of 10^{-6} M strongly inhibits thymidine incorporation. If the cells are left in R2956 longer than about 48 h, they begin to round up, detach from the surface of the dish and die. In experiments not shown, stimulation of precursor incorporation was generally not apparent before about 24 h of incubation, a lag period compatible with many steroid hormone effects[8].

This stimulation of precursor incorporation was reflected by an overall stimulation of net protein synthesis and cell division as shown in Table 2. 5-α-Dihydrotestosterone (10^{-6} M) stimulated protein synthesis per dish, with differences apparent after 2 d in culture. After 8 d triplicate control dishes contained an average of 120,000 cells as compared with 172,000 cells in androgen stimulated dishes. Thus, 5-α-dihydrotestosterone (which unlike testosterone cannot be aromatised to oestrogen) is capable of stimulating cell proliferation significantly in this human breast cancer cell line *in vitro*. We ascribe the smaller increases in net protein synthesis and cell proliferation as compared with stimulation of precursor incorporation to the fact that the experiments were performed in serum-free conditions which markedly inhibit cell growth.

Based on many previous studies on the mechanism of action of androgens[9,10] one would expect these cells to contain androgen receptor. Binding studies on cytoplasmic extracts using a dextran coated charcoal assay revealed high affinity limited capacity androgen binding molecules with affinity for both 5-α-dihydrotestosterone and testosterone. Scatchard analysis of the binding data revealed a class of receptors for 5-α-dihydrotestosterone of uniform affinity ($K_d = 7.5 \times 10^{-10}$ M, $r = 0.997$). When ^{3}H-testosterone was used instead, K_d was 1.5×10^{-9} M ($r = 0.958$). The

Table 1 Effects of various steroids on incorporation of thymidine and leucine into acid precipitable material in MCF-7 human breast cancer

Steroid	^{3}H-thymidine incorporation (d.p.m. $\times 10^{-2}$/µg protein/h $\pm$ 1 s.d.)	^{14}C-leucine incorporation (d.p.m. $\times 10^{-1}$/µg protein/h $\pm$ 1 s.d.)
Control	10.2 ± 1	7 ± 1.1
10^{-6} M 5-α-dihydrotestosterone	41 ± 1	16.7 ± 0.9
10^{-6} M 5-β-dihydrotestosterone	9.6 ± 1.9	7.8 ± 0.6
10^{-6} M Testosterone	19.6 ± 1.9	10.5 ± 1.1
10^{-6} M R2956	5.6 ± 0.3	Not done

Cells growing in monolayer cultures as previously described[6] were changed to serum-free Eagle's MEM After 24 h the medium was replaced with fresh serum-free medium. Hormones were added as 1,000–10,000 times concentrates in ethanol. After 48 h the cells were pulsed with labelled compounds and incorporation into macromolecules measured as previously described. Purity of 5-α-dihydrotestosterone and testosterone were checked by thin-layer chromatography[19].

Table 2 Effect of androgen on net protein synthesis in MCF-7 human breast cancer in tissue culture

	Time (d)				
	0	2	4	6	8
Control	25.8	30.8	35.5	38	43.1
$+10^{-6}$ M 5-α-dihydrostestosterone	25.8	35.7	40.4	46.4	53.8

Cells growing in monolayer were suspended with trypsin-EDTA solution, and plated in triplicate in 60-mm Petri dishes at a density of about 10^5 cells per dish. After 24 h, regular growth medium was charged to Eagle's MEM without serum. Hormone was added to 1/2 the dishes and at various times cells were collected and washed and total cellular protein per dish was estimated after sonication in water by the Lowry technique[20]. Values are in μg protein per 50-μl aliquot of homogenate.

approximately twofold lower dissociation constant for 5-α-dihydrotestosterone against testosterone suggests that the reduced compound may be the active androgen in these cells. The ability of these cells to convert testosterone to 5-α-dihydrotestosterone was examined directly by incubating cells in serum-free medium with ^{3}H-testosterone and examining the medium, total cell homogenate, and crude nuclear pellet prepared by differential centrifugation for formation of ^{3}H-5-α-dihydrotestosterone. Using methylene chloride to extract steroids, adding non-radioactive carrier steroids and running the organic phase on silica gel G plates using chloroform–methanol (98 : 2 v/v), we found counts migrating in the position of both 5-α-dihydrotestosterone and androstanediol in both the extracted media, cell homogenate and crude nuclear pellet. At 4 h, we estimated conversion to be 13.7%. This suggests that the cells do contain 5-α-reductase activity and that testosterone itself may be the precursor androgen in these cells *in vivo*. The cells rapidly metabolise 5-α-dihydrotestosterone to androstanediol and other more polar products. The description of these metabolic conversions will form the subject of a later communication but clearly the cells contain not only 5-α-reductase activity but 3-ketoreductase activity as well. In an experiment in which the number of binding sites for both compounds was quantified on a single cytosol preparation, specifically bound 5-α-dihydrotestosterone and testosterone differed by less than 5%. On most samples tested, about 50 femtomol 5-α-dihydrotestosterone were bound per mg of cytoplasmic extract.

Table 3 Ability of various steroids to displace either ^{3}H-5-α-dihydrotestosterone or ^{3}H-17-β-oestradiol from receptor in MCF-7 human breast cancer

Competitor	Molar excess	^{3}H-oestradiol % bound	^{3}H-5-α-dihydro-testosterone % bound
Control (no competitor)		100	100
17-β-Oestradiol	2	13	91
	20	8	62
	200	2	22
Nafoxidine	20	56	86
	200	19	80
	2,000	14	57
5-α-Dihydrotestosterone	2	100	11
	20	97	2
	200	98	1
R2956	20	97	17
	200	96	9
	2,000	97	4
Progesterone	20	87	73
	200	91	41
	2,000	93	34
β-Dihydrotestosterone	20	N.D.	85
	200		88
	2,000		92

Cytosols from MCF-7 human breast cancer were incubated with either 5×10^{-9} M ^{3}H-17-β-oestradiol or ^{3}H-5-α-dihydrotestosterone with or without the presence of excess unlabelled competitor as noted. After 18-h incubation at 0 °C specifically bound steroid was assayed[21]. Results are expressed as percentage of specifically bound tritiated steroid as compared with no competitor.

To demonstrate unequivocally that androgens were binding to a unique receptor site different from the oestrogen receptor also found in these cells[6,11], the cross competition studies outlined in Table 3 were performed. As shown, when ^{3}H-oestradiol is incubated with cytosol and presumably bound to oestrogen receptor, oestradiol and an antioestrogenic compound (Nafoxidine) can nearly completely displace the label from receptor sites whereas androgens and the antiandrogens (R2956) do not displace oestradiol. Conversely, when ^{3}H-5-α-dihydrotestosterone is incubated with cytoplasmic extracts and the ability of various steroids to displace androgen from receptor is examined, nearly the reverse results are obtained. While unlabelled oestradiol shows some ability to compete with ^{3}H-5-α-dihydrotesterone for binding a Nafoxidine has little binding affinity. Potent androgens easily displace labelled ^{3}H-5-α-dihydrotestosterone and antiandrogens also show activity. Progesterone displaces ^{3}H-5-α-dihydrotestosterone from its receptor but does not displace ^{3}H-oestradiol. 5-β-Dihydrotestosterone does not displace ^{3}H-5-α-dihydrotestosterone from receptor and, as shown in Table 1, does not promote thymidine or leucine incorporation. Thus, these data strongly suggest separate receptors for oestrogen and androgen in these cells.

The demonstration of androgen responsiveness of this cell line *in vitro* provides a rational basis for the response of at least some patients with metastatic breast cancer to adrenalectomy[12,13] since this ablative procedure may remove weak androgen precursors such as dehydroepiandrosterone and androstenedione which can be converted by some human breast cancer to more potent androgens[14]. Furthermore, since some human breast cancers may be responsive to androgen, it is possible that an examination of human tumours for androgen receptors may help with therapy of some patients, much as oestrogen receptor studies have appreciably improved therapeutic decision making in breast cancer[15]. We[16] and others[17,18] have described androgen receptors in some fresh human tumour samples but their value in guiding therapy remains to be seen.

Finally, the existence of a human cell line showing severalfold stimulation by androgens in a defined system, free from the effects of other trophic hormones should provide a useful reagent for the further study of the mechanism of androgen action.

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Nerve growth factor-induced process formation by cultured rat pheochromocytoma cells

NERVE growth factor (NGF) is a chemically characterised polypeptide which affects the development of the sensory and sympathetic nervous systems and is required for maintenance of sympathetic neurones[1-3]. Its mechanism of action and its precise role in embryogenesis are unknown.

NGF promotes neurite outgrowth from embryonic sensory or sympathetic ganglia both *in vitro* and *in vivo*[1-3]. This phenomenon has provided the basis of much NGF research, and is utilised in the most common NGF bioassay[4]. Some cells in cultured human neuroblastomas, but not in mouse neuroblastomas, also respond to NGF[5,6]. Homogeneous populations of replicating NGF-responsive cells, however, have not been available for study. We describe here NGF-induced process formation by a transplantable rat pheochromocytoma in cell culture.

Tumour cells were obtained from a previously reported noradrendine-producing adrenal pheochromocytoma[7,8], which was passaged subcutaneously in New England Deaconess Hospital white rats. The tumours were excised aseptically, rinsed in several changes of Ca^{2+} and Mg^{2+}-free phosphate-buffered saline, and mechanically dissociated by trituration with a Pasteur pipette. Dissociated cells were suspended in Dulbecco's modified Eagle's medium (Gibco) which was supplemented with 10% foetal bovine serum (Flow Laboratories), penicillin (50 U ml^{-1}) and streptomycin (50 µg ml^{-1}) and were plated on polystyrene tissue culture dishes (Falcon) which in some cases were coated with air-dried rat tail collagen[9]. Cultures were maintained at 37 °C in a water-saturated atmosphere of 92% air and 8% CO$_2$. The identity of the cultured cells as pheochromocytes was confirmed by electron microscopy and formaldehyde-induced fluorescence[8] (Fig. 1*e* and *f*).

Plating efficiencies (from inocula of 250,000 cells per 35-mm dish) were determined by counts of trypsinised cells or by measurement of total protein or absorbance (260 nm) of cells dissolved in 0.1 N NaOH. Process outgrowth was quantified by direct counts of cells or clumps of cells with processes, and was studied as a function of NGF concentration (2.5S mouse salivary gland NGF)[10] and of time. Some cells were grown in medium supplemented with hydrocortisone or corticosterone, at concentrations of 5–50 µg ml^{-1}.

NGF (50 ng ml^{-1}) produced an increase in plating efficiency from 50% to nearly 100% for freshly dissociated cells plated directly on plastic. On the collagen substrate, however, nearly identical plating efficiencies (~ 100%) were obtained with or without NGF. The cells had a marked tendency to grow in clumps, particularly when plated on collagen.

Short processes (20–30 µm) were evident within 24 h of plating, and thereafter elongated progressively. By 24 h *in vitro*, significantly more cell clumps with processes were demonstrable in cultures with NGF than in controls (Fig. 2*a*). By 1–2 weeks, twenty times as many process-forming clumps were present in the NGF-containing cultures (Fig. 2*a*). The actual difference between numbers of cells with processes was many times greater, but could not be quantified because individual cells often became obscured in rounded clumps (Fig. 1*d*). NGF-induced process formation followed a dose–response curve identical to that observed with primary cultures of sympathetic neurones[11], with concentrations of NGF as low as 0.5 ng ml^{-1} (2× 10^{-11} M) eliciting a maximum percentage of responding clumps after 24 h of treatment (Fig. 2*b*). Neither hydrocortisone nor corticosterone affected process formation.

Processes formed in the presence of NGF tended, after a

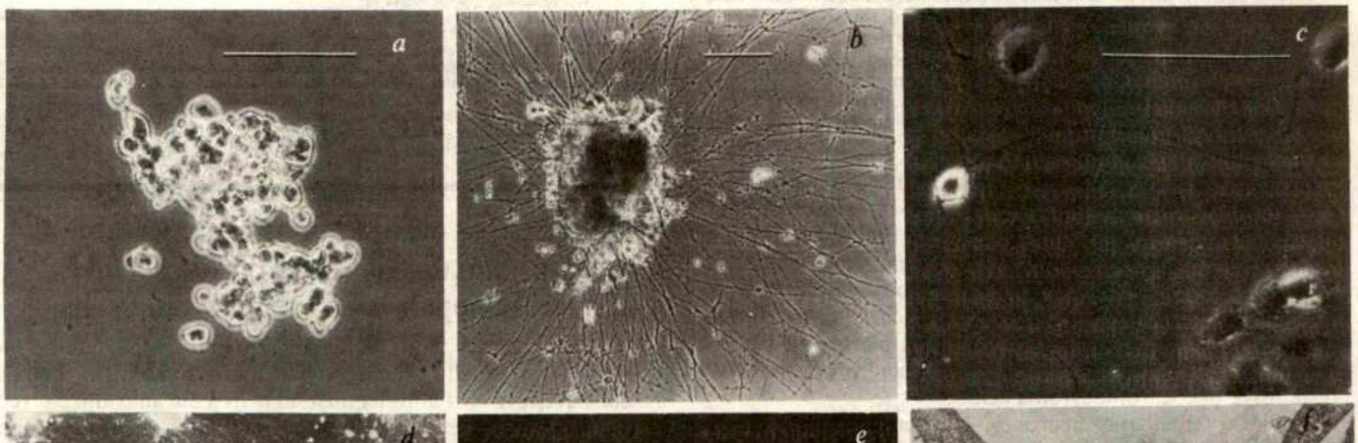

Fig. 1 Micrographs of cultured pheochromocytoma cells. *a* and *e*, No NGF; *b*–*d* and *f*, with NGF (50 ng ml^{-1}) in culture medium for 10 d. *a*–*c*, Unfixed, phase-contrast illumination; *d*, unfixed, dark-field illumination; *e*, formaldehyde-induced histofluorescence of catecholamines; *f*, electron micrograph showing chromaffin granules and part of a process. All cells were grown on a collagen-coated surface. The bar represents, in *a*–*e*, 100 µm, and in *f*, 1 µm.

week *in vitro*, to become longer (to > 500 μm) and more branching than in untreated cultures (< 50 μm), and many had varicosities (Fig. 1*a–d*). Such pheochromocytoma processes resemble those produced by sympathetic neurones in primary cultures, particularly with respect to their fineness of diameter and tendency to form fascicles[12,13] (Fig. 1*b–d*). Fasciculation, in our experience, is not shown by fibres from cultured murine neuroblastoma cells. Electron microscopy (Fig. 1*f*) revealed processes emerging from cells with typical noradrenaline-type chromaffin granules, and the proximal segments of these processes were comparable with those in similar regions of sympathetic neurones[14].

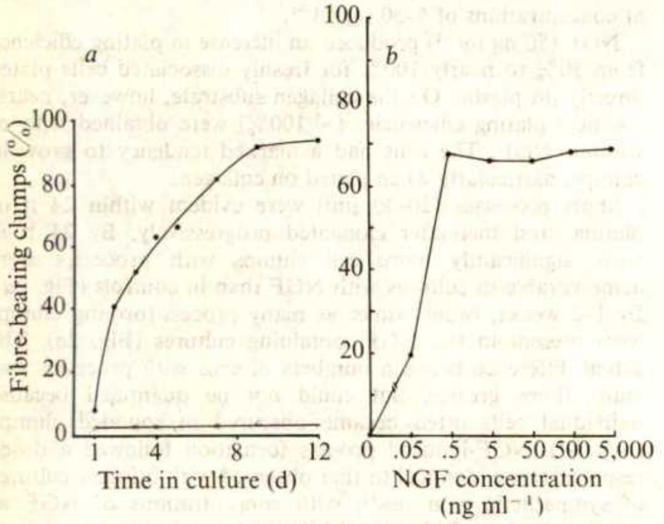

Fig. 2 Proportion of fibre-bearing cell clumps observed in cultures (*a*) at various times after plating in the absence (○) or presence (●) of NGF (50 ng ml^{-1}); (*b*), 24 h after plating in the presence of various concentrations of NGF. Cells in (*a*) were from a freshly dissociated tumour while those in (*b*) had been maintained previously for 2 weeks *in vitro* in the absence of NGF. Collagen-coated culture dishes were used. Counts were made in each case by examining the cultures with an inverted phase microscope (×200 magnification) and scoring the proportion of clumps from which processes (> 20 μm) extended. At least 300 cell clumps were scored for each count.

Tumour cells survived *in vitro* without NGF for at least 2 months. When NGF was then added to these cells, a larger percentage showed processes within 24 h than when freshly dissociated cells were treated with NGF. Although NGF-responsive cells were able to propagate, they grew extremely slowly. After approximately a month *in vitro*, cultures of pheochromocytoma cells started to become overgrown with epithelial-like and fibroblastic cells which did not produce catecholamines, or show NGF effects on plating efficiency or morphology. We are attempting to clone and propagate NGF-responsive pheochromocytoma cells.

Unlike sympathetic neurones, pheochromocytes are not generally thought to produce processes in response to NGF or to be affected by antibody to NGF[15,16]. Neither normal nor neoplastic pheochromocytes have long processes *in vivo*, in spite of the probable ubiquity of NGF produced by fibroblasts[17]. Process outgrowth, however, has been described from explants of human foetal adrenal medulla[18] and from cultured human pheochromocytoma cells in one of two studies[19,20]. We have now shown that this phenomenon, in a rat pheochromocytoma, is inducible by NGF. Process formation that occurs in cultures in the absence of added NGF might reflect another mechanism, or might be attributable to fibroblast NGF or NGF synthesised by pheochromocytes themselves.

One interpretation of our findings is that pheochromocytoma cells exhibit properties of both pheochromocytes and neurones as a manifestation of neoplasia, having partially regained the pluripotency of a more primitive neural crest cell. Another possibility is that all pheochromocytes have some capability to form long processes when removed from the presence of inhibitory substances which act *in vivo*. It has been postulated that glucocorticoids promote differentiation of foetal sympathoblasts into catecholamine-storing cells without processes[21,22]. We were unable to inhibit NGF-induced process formation in the rat tumour cultures with high concentrations of hydrocortisone or cortiscosterone, suggesting that factors other than glucocorticoids account for the absence of processes on these tumour cells *in vivo*.

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Quantitative evaluation of concanavalin A receptor site distributions on the surfaces of specific populations of embryonic cells

MORPHOGENESIS and malignant invasion seem to be dependent on cellular migration and changes in cellular adhesiveness[1–3]. Plant lectins such as concanavalin A (con A) have been extensively used to study the nature and distribution of lectin receptor sites on the surfaces of normal, embryonic and tumour cells. These sites may be involved in controlling cellular migration and adhesiveness[2,4,5].

Embryonic cells, like malignant cells, and other migratory cell types, are agglutinable with con A[2,6–8]. We have recently shown[9] that only the migratory population of sea urchin embryonic cells (micromeres) are agglutinable with con A and exhibit con A-induced capping of receptor sites[10]. Non-migratory embryonic cell types (macromeres and mesomeres) do not exhibit this increased con A receptor site mobility and are not significantly agglutinable with con A[10]. Thus, there seems to be a direct correlation between con A receptor site mobility[10–15] and cellular migratory activity.

Using microfluorometry[16,17], we have quantitatively examined the topographical distribution of con A receptors on agglutinable and less agglutinable cell types obtained from the sea urchin embryo. Using fluorescein isothiocyanate–concanavalin A (FITC–con A) we have measured the relative fluorescence intensities of four distinct areas (Fig. 1*a* and *b*) on the surfaces of individual cells of the agglutinable and less agglutinable types. This technique facilitates quantification of fluorescence topography of con A receptor sites on the surfaces of specific populations of embryonic cells.

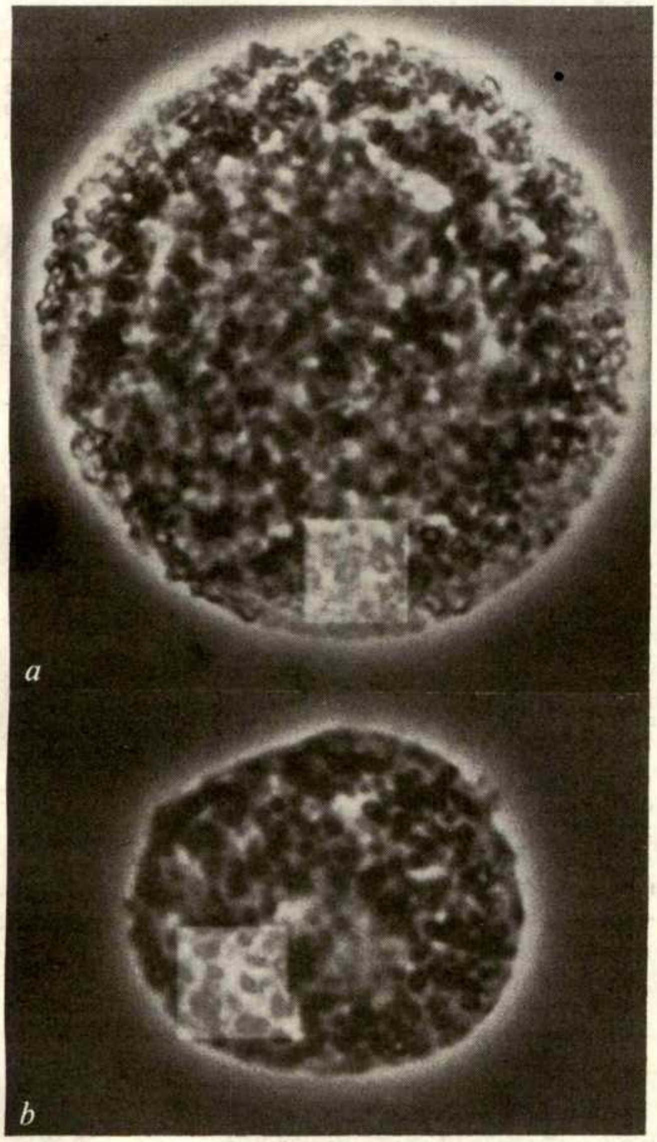

Fig. 1 Specific populations of embryonic cells showing measuring area. A sample containing cells of both populations that have been treated with FITC–con A (750 μg ml) and fixed in 4% formaldehyde as described in the text. An individual cell, of either type, was brought into focus by phase-contrast illumination before photoexcitation to limit fluorescence fading to a minimum. At this time diameter measurements were taken, the surface area calculated and entered in Table 1. The measuring field diaphragm for fluorescence quantitation was then adjusted to cover an area of 56.25 μm². Thereafter, the only light reaching the photomultiplier was that which was included by the measuring area only. To determine the topographical distribution of FITC–con A on the surface of individual cells, the fluorescence intensity of four distinct areas comprising 56.25 μm² were measured. The source for fluorescence excitation was from an XBO 150 W, 20 V Xenon lamp (Osram) powered by a Leitz power supply rated at ±0.1% constant voltage supply. The following light filters were used: excitation filter 5 mm BG12; red suppression filter 4 mm BG38; and suppression filters K495 and K510. The photomultiplier used was an EMI 9558 with photocathode type S20. The high tension supply unit was set to deliver 1.1 kv to the photomultiplier, the digital picoammeter set at a display rate of 1 s^{-1}. (ref.17). *a*, Macromere with measuring area (phase contrast ×1,625); *b*, micromere with measuring area (phase contrast ×1,625). All measurements of fluorescence intensity have been represented in Table 1 and Fig. 2.

Dissociated cells obtained at the 32/64 cell stage of developing sea urchin embryos (*Stronglycentrotus purpuratus*) were used. The procedures for preparation of the embryos and the dissociation into single cells have been described previously[9]. Dissociation of embryos at this cell-stage yields three cell types which are easily distinguishable by size: micromeres, mesomeres and macromeres. Of these, only the micromeres are migratory and significantly agglutinable by con A[9]. After dissociation into single cells the cell suspensions were layered on a 5–15% Ficoll gradient to separate out any cell debris, unfertilised eggs or undissociated embryos. The dissociated cells were removed from the gradient and washed twice in calcium and magnesium-free seawater (CMF–SW) containing 1% Ficoll. An appropriate preparation was then made to include all cell types at a total concentration of 8×10^6 cells ml^{-1} of CMF–SW. FITC–con A containing no free FITC and supplemented with 0.001 M calcium chloride was obtained from Miles–Yeda Laboratories. A sample (1 ml) of the cell suspension was treated with 750 μg ml^{-1} of FITC–con A, incubated at 17 °C for 10 min, followed with 4% formaldehyde (prepared in CMF–SW containing 1% Ficoll) fixation at 4 °C for 20 min. The cells were then washed with CMF–SW containing 1% Ficoll, mounted on slides and observed under incident illumination with a Leitz Orthoplan phase contrast fluorescence microscope equipped with a Leitz-MPV photometer fitted with an adjustable measuring diaphragm.

The fluorescence intensity of 10 micromeres and 10 macromeres was measured [17] and the mean recorded in Table 1. Overall, the macromeres had a mean fluorescence intensity (MFI) significantly higher than the micromeres ($P < 0.025$, Student's t test). When the MFI was calculated according to surface area (assuming a smooth sphere), the micromeres displayed greater fluorescence per unit area, but this difference was not significant at the 0.05 level. Although the micromeres were the highly agglutinable cell type, they did not significantly bind more FITC–con A per unit area than the less agglutinable macromere population.

Next we measured the fluorescence intensity of four distinct areas of 10 individual cells of each type to measure quantitatively any distributional differences of surface bound FITC–con A. The results of these measurements (Table 1 and Fig. 2) indicate that the micromeres displayed

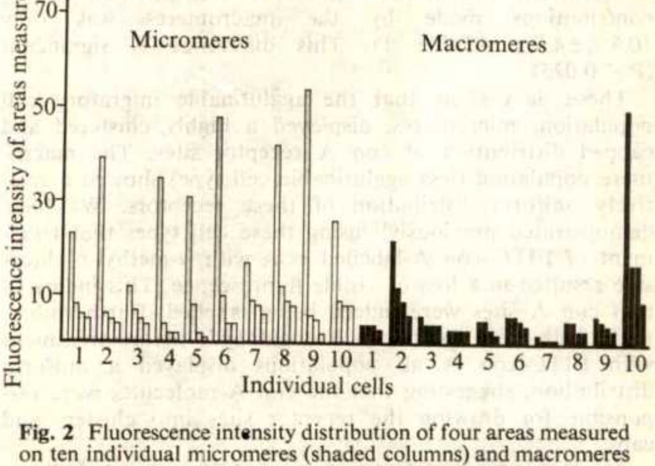

Fig. 2 Fluorescence intensity distribution of four areas measured on ten individual micromeres (shaded columns) and macromeres (solid columns). Abscissa: ten individual cells of both types. Ordinate: fluorescence intensity (%) of areas measured was derived from (see legend to Table 1):

$$\frac{\text{Intensity for each area measured}}{\text{Fluorescence intensity of individual cell}}\times 100$$

Table 1 Quantitative comparison of fluorescence topography displayed on the surfaces of specific populations of embryonic cells treated with FITC–con A

Cell type	Mean surface area (μm²)	MFI*	MFI per surface area (μm²)	MFI per area measured	MFI of areas measured excluding cap areas	MFI of cap areas measured only	Fluorescence intensity contribution by cap areas to MFI
Micromere	1,052.35 ±101.91‡	55.62 ±11.64	0.058 ±0.014	7.23 ±1.82	3.05 ±0.44	19.77 ±5.66	32.2% ±4.3%
Macromere	3,397.97 ±307.85	130.32 ±33.09	0.038 ±0.008	5.97 ±0.91	5.00 ±0.80	8.93 ±2.64	10.8% ±4.7%

*For measuring the mean fluorescence intensity of 10 cells of each type, the measuring field diaphragm was adjusted to provide an area which measured the fluorescence of only one cell. Corrections for fluorescence contributed by the background during these measurements have been made. For measurement of background fluorescence, three empty areas adjacent to the cell which had been previously quantified were measured, and the mean subtracted from the total fluorescence intensity. Units of fluorescence intensity are in terms of 10^{-8} A.

†An area measured for each individual cell of both populations was 56.25 μm².

‡Standard error.

an uneven distribution of receptor sites as judged by the high fluorescence intensity in one area compared with the other three areas measured. The concentration of FITC–con A to one area of the cell indicates a highly clustered or capped distribution, by both quantitative measurements, and visual observations. The macromeres displayed a relatively even distribution of fluorescence except for two cells which seemed to be clustered or capped (Fig. 2) when measured quantitatively, but not when observed visually. These quantitative measurements can therefore detect distributional differences of surface bound FITC–con A that would otherwise be left undetected by visual examination.

The MFI of all areas measured of individual cells comprising both populations showed that an area on a micromere was 1.35 times brighter than that of a macromere. But when the brighter areas or capped regions were omitted from the calculations, the macromeres had a higher MFI per area measured than micromeres. This indicates that the clustered or capped regions of the micromeres contributed significantly (52%) to the MFI per area measured, whereas the contribution made by the brightest areas to the MFI per area measured for the macromeres was only 16%. Furthermore, the MFI capped regions of micromeres and the brightest areas of non-capped regions of macromeres (including the areas of the two capped macromeres, Fig. 2) indicated that capped regions of the micromeres had a significantly ($P<0.005$) greater MFI than macromeres (Table 1). This suggests that the micromeres have areas on their surface membranes with a considerably greater con A receptor site density than macromeres. Comparison of the fluorescence intensity contribution made by the capped areas to the MFI of 10 cells of each cell type showed that the capped areas of the micromeres made a significant contribution of 32.2% ±4.3%, whereas the contributions made by the macromeres was only 10.8% ±4.7% (Table 1). This difference is significant ($P < 0.025$).

These data show that the agglutinable migratory cell population, micromeres, displayed a highly clustered and capped distribution of con A receptor sites. The macromere population (less agglutinable cell type) showed a relatively uniform distribution of these receptors. We have demonstrated previously[10] using these cell types that treatment of FITC–con A-labelled cells with α-methyl-D-glucoside resulted in a loss of visible fluorescence. This indicated that con A sites were indeed being labelled. Furthermore, if the cells were fixed with formaldehyde before treatment with FITC–con A all populations displayed a uniform distribution, suggesting that the con A molecules were responsible for drawing the receptor sites into clusters and caps.

The data obtained here using microfluorometry indicate that (1) the micromeres, being the more agglutinable cell type, do not bind significantly more FITC–con A per unit area than the macromeres and (2) con A-induced clustering or capping is significantly higher in the micromeres as judged by the higher variation in fluorescence intensity of the four areas measured of each individual cell (Fig. 2) and by the fact that the fluorescence intensity contributions to the MFI of 10 cells by the capped areas in micromeres was higher than that of macromeres (Table 1).

We have shown that quantitative fluorescence topographical measurements can be used to determine con A receptor site distribution on individual embryonic cells of specific populations.

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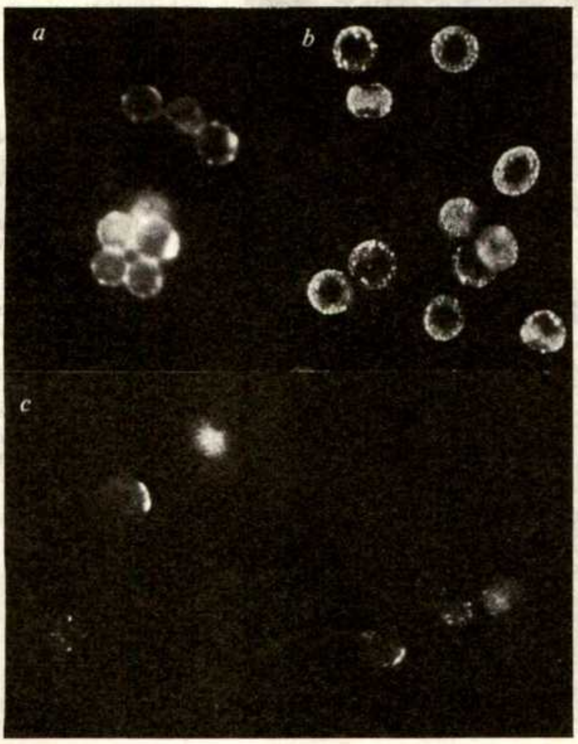
Redistribution of surface-bound polyelectrolytes on erythrocytes

REDISTRIBUTION of the largely diffuse binding sites on the cell surface of lymphocytes and many other cell types into clusters, or into highly polarised single aggregates or caps[1], is induced by interaction with divalent or polyvalent ligands such as antibodies, lectins or ligand-conjugated macromolecules[2]. Capping has been used as evidence for the fluid mosaic model of membrane structure[3] and it has been observed in various cell types, it may be a general property of animal cells[4]. An interesting exception has been the cell surface of the adult human red blood cell, which does not show clustering or capping with antibodies and lectins[5]. We have now demonstrated redistribution of fluorescent labelled polycations on the surface of erythrocytes fixed with glutaraldehyde or formalin when polyanions are added.

Human erythrocytes were washed four times with phosphate-buffered saline (PBS) *p*H 7.4, and twice with PBS containing 4% human serum albumin (PBS–4% HSA). Erythrocytes were fixed in 100 volumes of 1.5% formaldehyde in PBS for more than 3 weeks or in 2.5% glutaraldehyde in PBS containing 2mM $CaCl_2$ for 2 h at room temperature. Fixed erythrocytes were washed as intact erythrocytes, and suspended in PBS–4% HSA. Fluoresein-conjugated protamine and polylysine were prepared from fluorescein isothiocynate (FITC, Sylvana): 100 mg protamine sulphate (uridine sulphate (PS) given by Nordisk Insulin Laboratorium, Copenhagen) or polysine (polysine HBr (PL) molecular weight 37,200, Miles–Yeda) in 7 ml carbonate-buffered saline, *p*H 9.5 was added to 0.25 mg FITC in 1 ml of acetone and incubated for 30 min at room temperature. PS–FITC and PL–FITC were purified by reprecipitation in the cold and gel filtration on Sephadex G-25.

In the presence of HSA, intact erythrocytes were labelled with PS–FITC and PL–FITC within a few minutes and uniformly diffuse labelling was observed on 20–30% of them. Erythrocytes fixed with formalin or glutaraldehyde were 100% labelled in the same homogeneous way as the labelled intact cells. The covering of polycations was removed by one or two washings with PBS–4% HSA after which no fluorescent labelling was seen, but in the incubation medium the fluorescent surface coating was stable and unaltered after 24 h at room temperature. Incubation of erythrocytes in PBS without HSA gave immediate haemolysis with PL–FITC and slower haemolysis with PS–FITC. Red cell ghosts produced by polycation interactions, hypotonic conditions[6] or addition of minute amounts of saponin exhibited patching as distinct, rather uniform spots in the presence of PS–FITC and PL–FITC (Fig. 1*a*).

Fig. 1 *a*, Membrane fluorescence of human erythrocyte ghosts suspended in PBS 10 min after addition of PL–FITC (100 μg ml^{-1}) at room temperature. *b*, Intact human erythrocytes in PBS–4% HSA first incubated for 20 min with PS–FITC (100 μg ml^{-1}) 2–5 × 10^7 cells per ml and subsequent addition of dextran sulphate (1.0 mg ml^{-1}). *c*, Glutaraldehyde-fixed human erythrocytes as in (*b*). Cells were examined with a Leitz Ortholux fluorescence microscope fitted with a HBO 200-W mercury arc lamp and BG12 + UG1 excitation filters.



The uniformly distributed fluorescence label of polycations observed in both intact and fixed erythrocytes was redistributed after subsequent addition of the polyanion dextran sulphate (molecular weight 5 × 10^6, Pharmacia). Figure 1*b* and *c* shows the effect of dextran sulphate in a final concentration of 1 mg ml^{-1} on intact and fixed erythrocytes respectively, previously treated with PS–FITC. Immediately after addition of dextran sulphate small, very fine fluorescent dots were seen evenly dispersed over the entire surface.

The brilliantly fluorescent spots grew larger, and after 1 h at room temperature they had merged into a few large patches and after a further 2–3 h many cells had only one or two spots, in some cases reminiscent of capping.

Redistribution of membrane components is generally used as evidence for the fluid mosaic model of membrane structure. Redistribution has also been described[7] in the external membrane surface, where it is independent of membrane lipid fluidity. This concept is supported by our results showing that erythrocytes covered with a layer of polycations will, irrespective of whether the cell membrane is intact or immobilised, exhibit clustering and patching when interacted with a polyanion. Similarly, Davis[8] showed that the addition of multiple antibodies to the lymphocyte surface physically alters the distribution of H–2 isoantigens. If, for example, ferritin-conjugated alloantibody is bound to the lymphocytes, it is uniformly distributed over the cell surface. Subsequent interaction with ferritin antibodies causes coalescence of the ferritin conjugates into discrete zones of the cell surface. Multiple layer techniques for immunological detection of cell surface sites may therefore result in redistribution.

Interaction of polybases with erythrocyte ghosts has been studied by Katchalsky *et al.*[9]. Electron microscopy revealed that the polybases join neighbouring parts of the membrane (surface agglutination), resulting in distortions and eventually the formation of holes. This would explain the haemolytic effect of PL–FITC and PS–FITC. The clustering of fluorescent spots in our experiments may be due to such surface agglutination.

The interaction of polyanions with surface-bound polycations leads primarily to the formation of polar bonds whereby reductions in repulsive potential occur. The clustering and merging of material presumably results from random movements of surface bound components of the cells. The extensive redistribution of surface-bound material due to interaction between the cell membrane and compounds with high binding affinity would explain the increased cellular uptake of albumin[10] and the increased permeability to albumin induced by protamine in Congo-red gelatine membranes[11].

This report calls attention to the possibility that redistribution of material bound to the cell surface can occur even when membrane proteins are immobilised. Observations of capping cannot therefore be taken as unequivocal evidence for lateral mobility of the membrane constituents.

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Erythrocyte morphology and clustering of fluorescent anti-A immunoglobulin

PLASMA membranes of many cells are fluid, with the protein and lipid components mobile in the plane of the membrane[1,2], as changing distribution patterns of fluorescent labelled lectins and antibodies bound to cell surfaces have shown[1–4]. Such observations have not been made with intact human erythrocytes, which are thought to be an exception[5–7]. Ferritin-labelled concanavalin A (con A)[8] and soybean agglutinin (unpublished results of C. Kuettner, A. Staehlin, and J.A.G.) have been shown to be randomly arranged on the membranes of intact erythrocytes. But some clustering of con A receptors on the membranes of intact erythrocytes was shown by freeze etching when con A-coated erythrocytes were treated with anti-con A antibodies[8]. We now report evidence for clustering of fluorescein-labelled anti-blood group A antibodies on the surfaces of intact human A erythrocytes associated with alteration of erythrocyte morphology.

Defibrinated anti-A serum from Dade was precipitated with 50% saturated ammonium sulphate, redissolved in phosphate-buffered saline (PBS) and dialysed at 4° C against PBS. The dialysed solution containing anti-A antibodies was mixed for 24 h at 4 °C with a solution of fluorescein isothiocyanate (FITC Isomer I, Sigma, about 5 mg FITC per 100 mg protein) in PBS with the *p*H adjusted to 9.0–9.5 by addition of trisodium phosphate. Free fluorescein was removed by dialysis against PBS at 4 °C in the dark for 1 week. The FITC conjugated anti-A (FITC–anti-A) solution was used without further dilution (anti-A haemagglutination titre of 1: 512). Fresh human erythrocytes were obtained in EDTA and washed four times with PBS before use.

To obviate any difficulty in interpretation due to cell agglutination, erythrocytes were prepared for fluorescence microscopy as follows. Single drops of washed erythrocyte suspensions in PBS (about 4% v/v) were applied to glass microscope slides for 1 h at room temperature[10]. Nonadherent erythrocytes were removed by three washes of the slides in PBS. Adherent erythrocytes were then incubated with FITC–anti-A for 2 h at room temperature. Excess reagent was removed by three more washes with PBS.

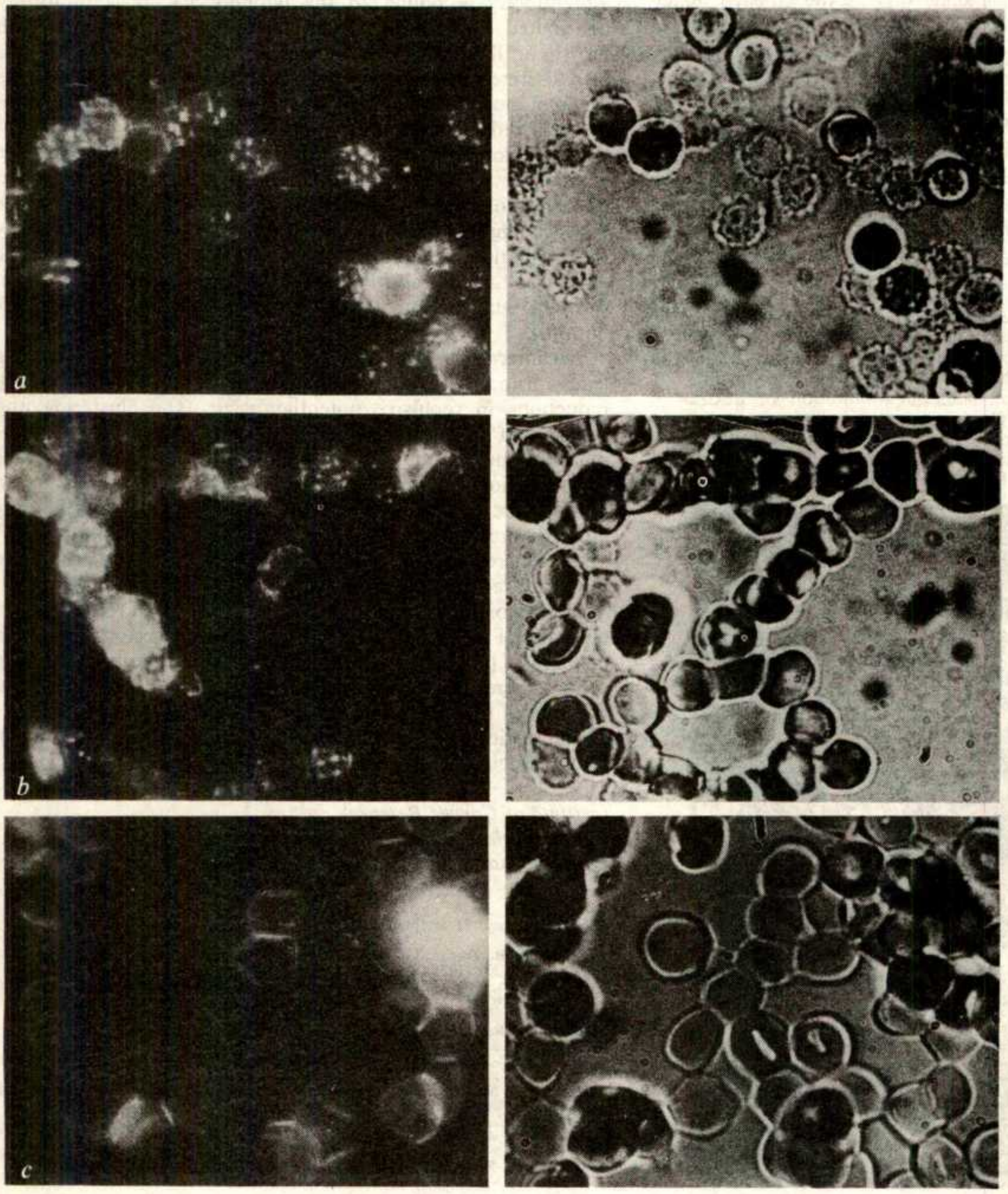

Fig. 1 Examples of fluorescent patterns obtained with human A-type erythrocytes stained with FITC–labelled anti-A globulin in several conditions. Fluorescent and phase photomicrographs of identical fields are adjacently placed. *a*, Cells which have been washed, stained and washed in PBS. Disk shape of cells converted to crenated form at staining step (line 1 in Table 1). *b*, Cells which have been washed and stained in PBS (like above to give crenates) and the morphology restored by final washes in PBS containing 1% BSA (line 2, Table 1). c, Cells which have been washed, stained and washed in PBS containing 1% BSA biconcave cell shape never was crenated (line 3, Table 1).

Table 1 Morphology and fluorescent patterns of FITC–anti-A-treated human erythrocytes

Morphology during preparative stages				Fluorescent pattern
Initial washes	FITC–anti-A	Final Washes	Final observation medium	
Rough disk (PBS)	Crenate (PBS)	Crenate (PBS)	Crenate (PBS)	Stippled
Rough disk (PBS)	Crenate (PBS)	Crenate (PBS)	Disk (B)	Stippled
Disk (B)	Disk (B)	Disk (B)	Disk (B)	Uniform
Rough disk (PBS)	Crenate (PBS)	Crenate (PBS)	Rough disk (V)	Stippled
Cup (V)	Rough disk (V)	Disk (V)	Disk (V)	Uniform
Crenate (S)	Rough disk (B)	Disk (B)	Disk (B)	Uniform

A disk is a cell with the usual biconcave disk shape. A rough disk is a discoid cell with a few rounded crenations. A crenate is a spherical cell with many fine spikey crenations. A cup is a hemispherical cell with a single exaggerate concavity. These first three morphologies represent stages of a spectrum of morphological changes usually referred to as the diskocyte–echinocyte transformation[9]. B, 1% BSA in PBS; V, 0.02 mM vinblastine in PBS; S, 0.005 M sodium salicylate in PBS. Fluorescent patterns are fully described in the text.

After addition of a drop of PBS and sealing of coverslips slides were examined with Zeiss and Leitz microscopes equipped with ultraviolet epi-illumination. PBS containing 1% bovine serum albumin (BSA, Metrix) or 0.02 mM vinblastine (Lilly) was substituted for PBS in some experiments.

The FITC–anti-A globulin labelled blood group A but not B or O erythrocytes. The specificity of the fluorescence was demonstrated by showing a reduction in fluorescence intensity by preincubation of erythrocytes with unlabelled anti-A but not with anti-B blood typing serum.

The fluorescent patterns were of two general types, designated uniform and stippled. Uniform fluorescence had a uniform intensity distributed over the entire erythrocyte; a rim of fluorescence was observed where the membrane was viewed tangentially. Stippled fluorescence was of greater intensity, discretely localised to multiple (14±4 per cell image) small areas about 0.5 μm or less in apparent diameter, superimposed on a dimmer uniform fluorescence of the erythrocyte surface. A tenfold dilution of the FITC–anti-A globulin reduced the total fluorescent intensity without altering the stippled pattern.

Erythrocytes washed, stained, washed and observed in PBS were extensively (finely) crenated and had stippled fluorescence, which was most intense on the tips of the spicules (Fig. 1*a*). The biconcave morphology of the crenated erythrocytes, which were labelled with FITC–anti-A, could be restored by addition of PBS containing 1% BSA[11]; stippled fluorescence was not reversible (Fig. 1*b*). Cells prepared with 1% BSA in PBS at every stage of preparation showed little or no crenation and had uniform fluorescence (Fig. 1*e*). The use of BSA was not specific, for similar results were obtained with a very low concentration of vinblastine (Table 1); 0.02 mM vinblastine restored the crenated erythrocyte to an irregular disk.

We next examined crenation by an agent other than the FITC–anti-A label (Table 1). Extensive precrenation of erythrocytes produced by exposure to 5 mM salicylate[12] in PBS, followed by reversion to disks by washing with 1% BSA in PBS before staining with FITC–anti-A containing 1% BSA gave the same uniform fluorescence seen in the absence of precrenation..

Our results indicate that the fluorescence pattern produced by FITC–anti-A on the surface of A erythrocytes is related to erythrocyte morphology. Stippled fluorescence occurs only when the A erythrocyte becomes extensively crenated in the presence of the anti-A antibody[13]. Crenation can be minimised and stippling prevented if BSA or vinblastine is present throughout the preparative procedure. But the stippled pattern is not reversed by BSA or vinblastine after labelling even though the morphology reverts from a crenated sphere to a disk.

We believe that the stippling is due to the movement of A antigenic sites on the erythrocyte membrane, during crenation and in the presence of FITC–anti-A, to form discrete clusters of antigen and fluorescent antibody at the tips of cellular spicules, so that fluorescence concentrates there. We have not been able to separate crenation from site clustering in the presence of anti-A globulin; precrenation did not lead to stippling if the erythrocyte was restored to a disk morphology before labelling with FITC–anti-A. Two studies provide some evidence for the movement of structure in intact erythrocyte membranes. Anti-con A antibodies caused clustering of previously randomly arranged con A on the erythrocyte surface made visible by freeze etching[8]. Chevelier's work indicates a moderate increase in the number of intramembranous particles per unit area at the apices of cellular spikes of crenated erythrocytes in the absence of erythrocyte–antibody interaction[14]; this supports the suggestion that crenation leads to the movement of membrane components. The irreversibility of the clustering after the reversal of shape to a more native form (Fig. 1*c*) could depend on the antibody cross linking of membrane sites. A reasonable interpretation of our results is that cytoskeletal elements of intact erythrocytes[15–19] generally restrict the mobility of receptors over the membrane surface. Crenation of the cell either frees membrane components from the restriction of movement or the crenation mobilises the cytoskeletal elements so that they direct the movement of the anti-A receptors to the apices of the crenates. Our experiments provide no evidence to aid the choice between these possibilities. The mechanism by which vinblastine prevents stippling is unclear. It might be a direct effect on cytoskeletal components[20], an alteration of membrane proteins[21] or a change in membrane fluidity.

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Accumulation of 1,2-diacylglycerol in the plasma membrane may lead to echinocyte transformation of erythrocytes

THE change in shape of erythrocytes from the normal biconcave disk to a spiculed sphere (echinocyte[1]) can be caused by an increased intracellular concentration of Ca^{2+} (refs 2 to 5). We have found that an immediate biochemical effect of raising intracellular $[Ca^{2+}]$ is to increase the production of 1,2-diacylglycerol and cause its accumulation in the plasma membrane. This change in membrane composition may alter the structure of the membrane and thus cause the change in shape. Energy-depleted cells also show increased 1,2-diacylglycerol and similar but less extreme morphological changes, even in the absence of Ca^{2+}.

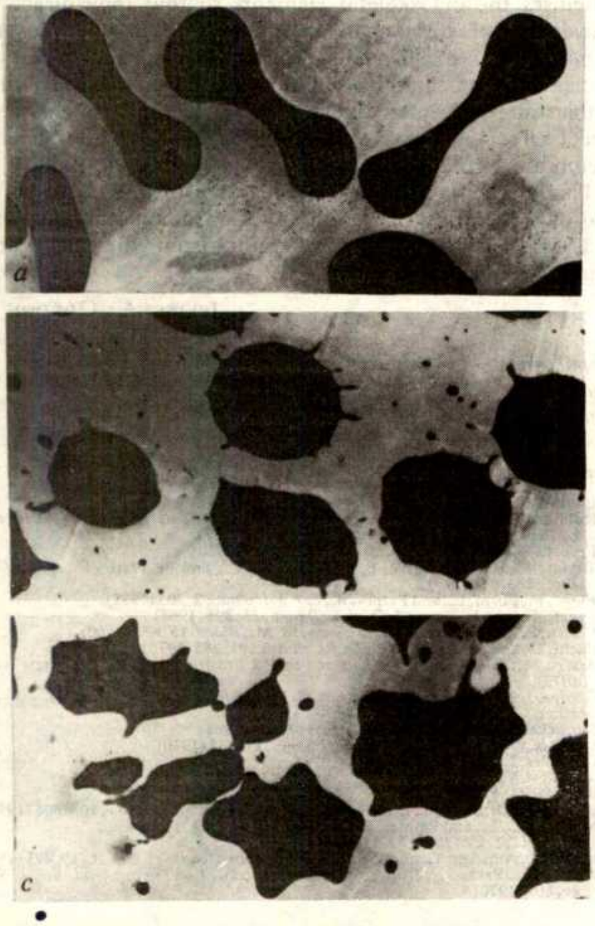
Washed human erythrocytes (2×10^9 ml^{-1}) were incubated at 37 °C for 1 h in HEPES–Ringer[6] containing 11 mM glucose and bovine serum albumin 1 mg ml^{-1} (Sigma), until the cells were uniform biconcave disks. Samples (1 ml) of the cell suspension were added to test tubes containing 1 μg of the divalent cation ionophore A23187 (ref. 7) and incubated for a further 1 h. The morphological changes leading to the echinocyte form[1-3] were complete within 1 h when glucose was available and within only 10 min in its absence (Fig. 1). The electron micrographs (for example, Fig. 1*b*) suggested that membrane microvesicles were pinched off from the microvillus-like membrane processes. This was confirmed by taking the low speed supernatant after sedimentation of ionophore-treated erythrocytes and subjecting it to high speed centrifugation: electron microscopy showed that the resulting pellet consisted mainly of membrane vesicles about 100 nm in diameter.

Lipids were extracted from control and ionophore-treated cells and chromatographed[6,8]. The most obvious change was the increased 1,2-diacylglycerol content of ionophore-treated cells (Fig. 2). Gas–liquid chromatography, carried out by Dr R. B. Watts, showed that ionophore-treated cells contained ~3.5 nmol of 1,2-diacylglycerol per 10^9 cells, compared with ~0.5 nmol per 10^9 control cells. In both cases the fatty acid composition of the diacylglycerol was similar to that of human erythrocyte phosphatidylcholine (D.A. and R.H.M., unpublished).

The total diacylglycerol production is greater than the increase noted above since in cells to which glucose was available much of the diacylglycerol generated in the early stages of ionophore treatment was converted to phosphatidate by diacylglycerol kinase, a very active enzyme in erythrocytes[9,10]. Consequently, in erythrocytes treated with A23187 for 20 min there was a tenfold increase in the incorporation of ^{32}Pi into phosphatidate (Table 1) and a 50% increase in the phosphatidate content of the cells. The identity of the radioactive phosphatidate was established by its chromatographic behaviour and by demonstrating that when subjected to mild alkaline methanolysis it gave glycerophosphate[10]. Other phospholipids were separated by thin-layer chromatography[10] and their phosphate contents were measured; no significant changes were observed in the concentrations of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol or sphingomyelin.

Cells treated with A23187 and a Ca^{2+}-chelator (EGTA) showed neither a change in shape nor any alteration in

Fig. 1 Electron micrographs showing the effect of calcium ionophore or energy depletion on human red cell morphology *a*, Normal cells; *b*, treated with A23187 for 1 h; *c*, cells incubated in medium free of Ca^{2+} and glucose for 20 h in the presence of iodoacetate, arsenate, deoxyglucose and EGTA (all 2 mM). Cells were fixed in suspension with 2% glutaraldehyde and stained with osmium tetroxide. Thin sections were prepared and examined in a Philips EM 301 electron microscope.



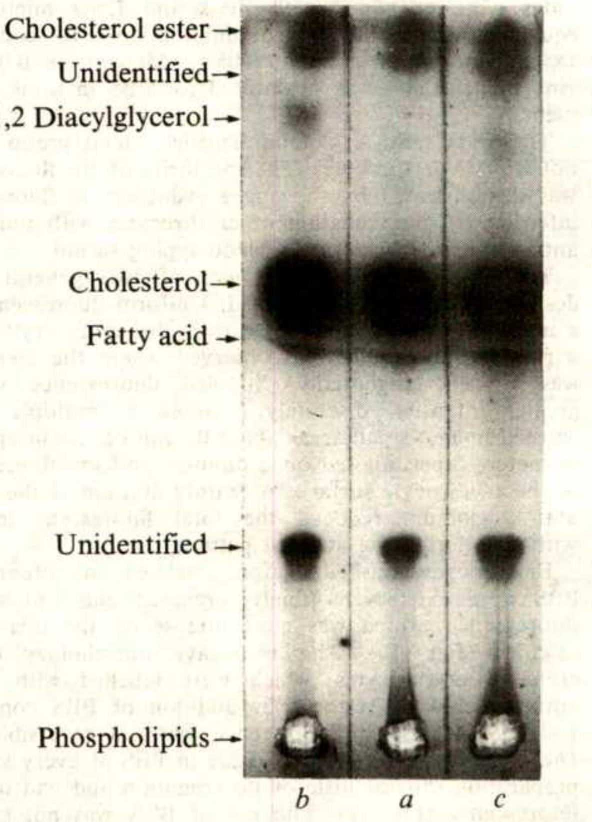


Fig. 2 Thin-layer chromatograms of neutral lipids from cells treated as in Fig. 1*a*, *b* and *c*. Lipids were separated on silica gel G plates using benzene–diethyl ether–ethanol–acetic acid (50:40:2:0.2) as described before[8]. Spots were made visible with iodine vapour and compared with standard lipids run on the same plates.

diacylglycerol or phosphatidate levels, but addition of Ca^{2+} in excess of the EGTA did initiate these changes, which therefore depended on entry of Ca^{2+} into the cells.

Experiments with isolated erythrocyte ghosts showed that diacylglycerol kinase was not activated by Ca^{2+}, confirming that phosphatidate synthesis was a result of increased availability of 1,2-diacylglycerol. Ca^{2+}-stimulated production of labelled phosphatidate was observed by Redman[11,12] in erythrocyte ghosts, but the possibility that this was secondary to diacylglycerol production was not considered.

The shape change of the ionophore-treated erythrocytes (with glucose) occurred after about 30 min, and at the same time as accumulation of 1,2-diacylglycerol. It occurred after the main period of phosphatidate labelling, suggesting that the cells could no longer phosphorylate diacylglycerol. This was confirmed when measurements of ATP levels in treated cells showed a 90% decrease in 30 min. This decrease, which was probably a result of the cell pumping out excessive intracellular Ca^{2+}, explains why glucose-deprived ionophore-treated cells were unable to phosphorylate diacylglycerol and were converted rapidly to the echinocyte form.

Table 1 Effect of A23187 on incorporation of ^{32}Pi into human red cell phosphatidate

	Radioactivity in phosphatidate (d.p.m.)
Control+Ca^{2+}	472
Control+EGTA	436
+A23187+Ca^{2+}	5,227
+A23187+EGTA	514

Values quoted are averages of triplicate determinations. Red cells (2×10^9) were incubated for 20 min at 37 °C either in complete HEPES–Ringer[6] containing 2.5 mM Ca^{2+} or in Ca^{2+}-free HEPES–Ringer supplemented with 2.5 mM EGTA. Lipids were separated by paper chromatography on formaldehyde-treated papers using *n*-butanol–formic acid–water (4:1:5). In this system phosphatidate runs close to the solvent front and ahead of the other phospholipids[10]. The spots containing phosphatidate were cut out, digested in 72% HClO$_4$ and their ^{32}P radioactivity was measured by Cerenkov counting[10].

The rapidity of the shape change in energy-depleted cells revived the possibility that lack of intracellular ATP may be sufficient to lead to the transformation of cells into echinocytes[5,13]. This idea is not easily disproved, since during energy depletion intracellular Ca^{2+} and diacylglycerol concentrations will tend to rise due to lack of intracellular ATP. The following experiment, however, suggests that diacylglycerol accumulation is the most important of these three factors. Erythrocytes were incubated at 37 °C without glucose in the presence of 2 mM iodoacetate, 2 mM arsenate and 2 mM deoxyglucose, either with Ca^{2+} (2 mM) or in its absence (2 mM EGTA), and their morphology and lipid composition were examined. After 20 h they appeared spherical under light microscopy, both with and without Ca^{2+}. Under electron microscopy they resembled ionophore-treated cells except that they were less regular in shape and showed fewer surface projections (Fig. 1). There was accumulation of 1,2-diacylglycerol, even in the absence of Ca^{2+}, but this was less marked than in ionophore-treated cells (Fig. 2), as expected from the smaller morphological effects. It presumably resulted from a process different from that triggered by Ca^{2+} influx into healthy cells, possibly phosphatidate phosphohydrolase activity; this activity exists in these cells[14] and is not influenced by Ca^{2+} (D.A. and R.H.M., unpublished).

Thus biconcave erythrocytes are converted to echinocytes if diacylglycerol accumulates in the cell membrane. The diacylglycerol can be produced either in the presence of increased intracellular Ca^{2+} (from phosphatidylcholine?) or during energy deprivation (from phosphatidate?). In both cases, accumulation of diacylglycerol, leading to membrane evagination and vesiculation, will occur mainly in the inner leaflet of the membrane, since the changes occur only in response to alterations in the internal Ca^{2+} or ATP levels. This might be an effect produced by diacylglycerol accumulation in the inner leaflet of the membrane, for the addition of diacylglycerol or ceramide to the outer leaflet leads to invagination and inward vesiculation[10]. In both situations membrane fusion occurs in the diacylglycerol-enriched membrane face, a result consistent with the observation that externally added diacylglycerol causes fusion of avian erythrocytes[15]. Production of diacylglycerol may also explain the observation that intracellular Ca^{2+} facilitates cell fusion[16]. Ca^{2+}-stimulated production of diacylglycerol is not confined to erythrocytes, for pig lymphocytes, when incubated with A23187, Ca^{2+} and ^{32}Pi, showed rapid labelling of phosphatidate (data not shown). Furthermore, during muscarinic cholinergic stimulation of pancreas, when intracellular [Ca^{2+}] increases, there is a pronounced rise in diacylglycerol concentration[17]. It therefore seems possible that Ca^{2+}-stimulated diacylglycerol production might have a role in various physiological situations where intracellular Ca^{2+} has been implicated in cell functions involving changes in plasma membrane topography, fluidity or fusibility[18–20].

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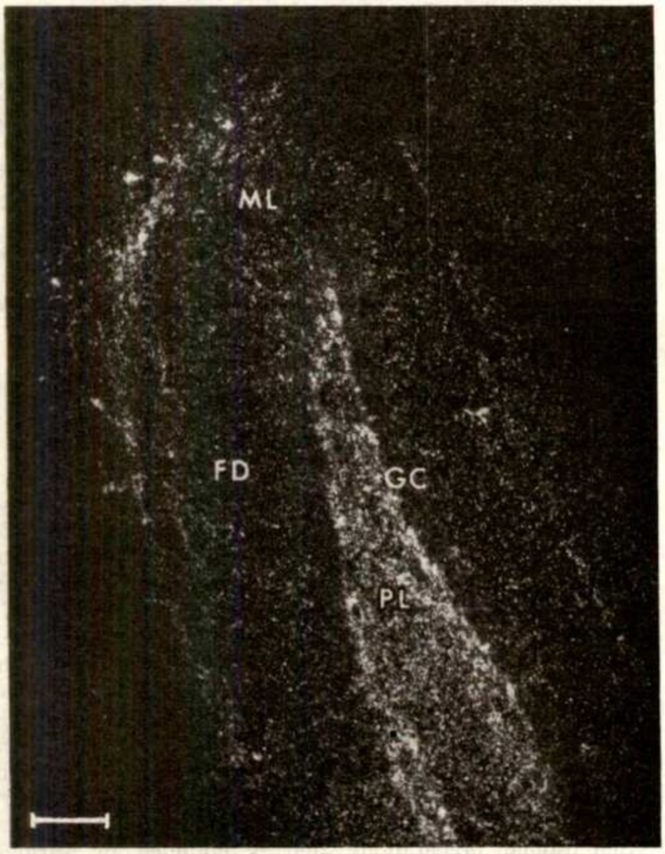
Autoradiographic localisation of α-bungarotoxin-binding sites in the central nervous system

SNAKE α-toxins have high specificity and affinity for nicotinic receptors, and have been used widely for the characterisation of peripheral acetylcholine receptors of electric organs and skeletal muscle. In some cases autoradiography has shown the extent and local distribution of bound neurotoxins[1–5]. Although α-bungarotoxin (α-BTX) does not pass the blood–brain barrier and therefore does not enter the central nervous system (CNS) to a measurable extent[6], central nicotinic acetylcholine receptors may bind α-toxins *in vitro*, and several investigators have used this toxin to characterise brain receptors[7–9]. Its specificity for central nicotinic receptors has been inferred not only from its peripheral action, but also from cytological and pharmacological observations: binding activity is highest in synaptosomal preparations, and is inhibited by various nicotinic drugs. Studies on the localisation of toxin-binding sites in brains, not so far reported, would be valuable, not only to corroborate neurotoxin specificity, but also to provide information on the regional distribution of nicotinic receptor sites in the CNS.

Using ^{125}I-α-BTX, we have found concentrations of binding sites in homogenates of whole brain comparable with receptor concentrations reported for the innervated rat diaphragm[10,11]: approximately 1.6 pmol per g of wet tissue in adult rats, and about 7.5 pmol per g of wet tissue in the newly hatched chick. Assays performed on individually dissected brain regions revealed marked local differences in concentrations of nicotinic receptor sites in both species; high values were repeatedly obtained for the colliculi, olfactory tubercle and hippocampus of the rat, and the retina and optic tectum of the chick, whereas other areas showed intermediate, or, as in the case of rat cerebellum, little if any binding activity (J.S., unpublished). These observations prompted a study of the distribution of α-toxin-binding sites in the central nervous system by means of light microscopic autoradiography.

Anaesthetised rats (approximately 200 g body weight) and chicks (1–7 d) were perfused with saline through the left ventricle. Brains were removed, frozen in methylbutanol at −70 °C and sectioned in a cryostat at −16 °C. Sections of 16 μm were collected on subbed slides and incubated at room temperature for 30–60 min in a solution of 0.1 M sodium phosphate, *p*H 7.4, 3×10^{-9} M ^{125}I α-BTX (approximately 10^{6} Ci mol^{-1} depending on the date of the experiment); this toxin concentration approximately equals the concentration of toxin-binding sites in whole brain. Control sections were preincubated with 10^{-7} M α-BTX or 10^{-3} M nicotine for 30–60 min after which the radioactive toxin was added. The slides were washed three times for 20 min each time in 0.1 M sodium phosphate, *p*H 7.4, fixed in 95% ethanol, rinsed with distilled water, and dried overnight at 40 °C. Dried slides were dipped in NTB 2 Nuclear Track Emulsion (Kodak), exposed at 4 °C for periods from 2 to 7 d, developed, stained with cresylviolet and coverslips placed in position. In spite of on-the-slide incubation with the radioactive toxin, low background levels were routinely obtained.

Fig. 1 Dark-field photograph of an autoradiograph of a sagittal section through the layer of polymorphic cells of the rat hippocampus formation. FD, fascia dentata; GC, granule cell layer; ML, molecular layer; PL, polymorphic layer. A section, 16 μm thick, was labelled as described in the text. Exposure time was 5 d. Scale line represents 200 μm. The inset shows the polymorphic layer of an adjacent section treated with nicotine before incubation with ^{125}I-α-BTX.



Autoradiography confirms the regional distribution of radioactive α-BTX measured by biochemical techniques and takes topographical analysis to the histological level. Figure 1 shows an example of the pattern of localisation within the hippocampal formation. Most label seems to be within the polymorphic cell layer of the fascia dentata and the thin strip in the outermost part of the stratum oriens of the hippocampus. The outer molecular layer, granule cells of the fascia dentata, Ammons pyramids and fimbria fornicis are unlabelled.

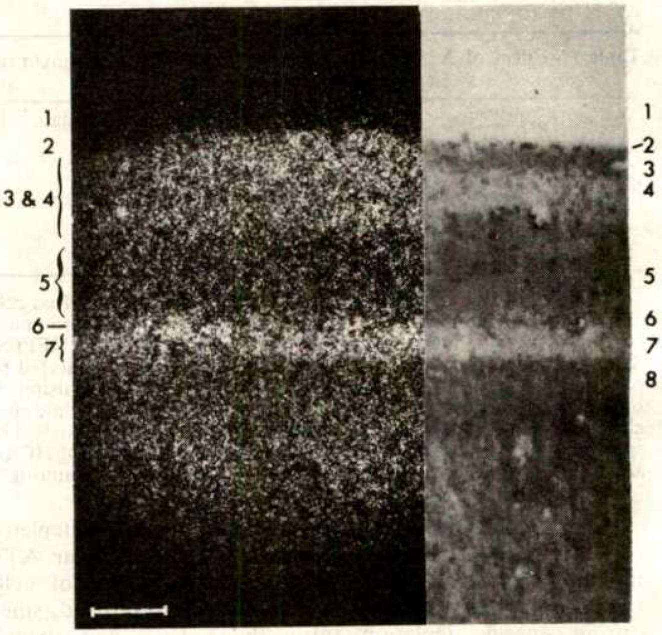


Fig. 2 Superficial layers of the optic tectum of the chick. Transverse sections show results of: Nissl stain, and acetylcholinesterase stain (modified Koelle method[19]) (shown on the right-hand side); and autoradiography with ^{125}I-α-BTX as described in the text (seen on the left-hand side). Exposure time was 2 d. Scale line represents 100 μm.

Homologous structures in rat and chick show similar uptake of label. Highest concentrations of toxin-binding sites occur in the optic tectum (see below), parabigeminal-isthmic nuclei, dorsal motor nucleus of the vagus, and nucleus geniculatus lateralis pars ventralis (external layer). Values are lower for thalamus, inferior colliculus and reticular formation of rat and chick, neocortex of rat and dorsal ventricular ridge of chick. A low, but significant level of binding is seen in the cerebellum whereas the caudate-putamen as well as various fibre tracts including optic, mammillo-thalamic and pyramidal tracts seem to be devoid of binding sites.

Usually, the toxin binds to areas known to contain high levels of acetylcholinesterase[12,13]. Exceptions are observed within certain laminated structures, as shown for superficial portions of the chick optic tectum (Fig. 2). Layer 1 lacks acetylcholinesterase as well as α-toxin-binding sites. But whereas the esterase is found chiefly in layer 5, and to a lesser degree in layer 3, nicotinic receptors seem to be concentrated mostly in layers 3, 4 and 7. Esterase is virtually absent from the latter two layers. Layer 7, which exhibits one of the highest toxin-binding levels of any brain area, contains no neurones, but radial dendrites of only the deeper-lying cells of layers 8–10. This suggests that nicotinic receptors are confined to selected segments of the dendrites.

Several regions known for their high esterase levels do not bind appreciable quantities of α-BTX. Presumably such areas contain muscarinic or other cholinergic receptors. Examples are the caudate nucleus, which according to physiological and biochemical studies contains muscarinic synapses in high concentration, and the cerebellum, which seems to lack muscarinic binding sites as well[14–17].

Also interesting is the high concentration of toxin-binding sites in the nucleus supraopticus. Nicotine is known to cause an antidiuretic effect by stimulating the hypothalamicohypophyseal system and thereby triggering the release of antidiuretic hormone[18]; conceivably the nicotinic receptor sites involved in this response are among those autoradiographically visualised in the hypothalamus.

The unique localisation of α-BTX reported here is further evidence for the hypothesis that the snake α-toxins bind to central and peripheral nicotinic acetylcholine receptors with essentially equal specificity. α-BTX labelled with ^{125}I has been used for an electron microscopic investigation of acetylcholine receptors in the neuromuscular junction[5]. We can therefore expect that this toxin will prove useful for the study of nicotinic synapses in the CNS as well, not only at the light microscopic, but also at the ultrastructural level.

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Slowing of ionic currents in the voltage-clamped squid axon by helium pressure

PRESSURE has been shown to affect the excitability of compound and single nerves[1–4]. The most consistent effect is a prolongation of the action potential, suggesting an influence of pressure on the kinetics of the excitation process. The physical biochemistry of pressure has been described in other systems, notably luciferin–luciferase[5], but similar analysis for nerve action potentials proves difficult because of the complexity of the process measured. By use of the voltage clamp[6] more direct assessment of effects on kinetic and other excitation parameters can be made with definition of pressure effects in Hodgkin–Huxley (HH) terms[7]. We now report the effects of pressure on the behaviour of the voltage-clamped squid axon.

Experiments were carried out in a pressure chamber[8] constructed from a commercially available high pressure pipe cross. Squid axons were dissected and mounted in an axon bath module. Incorporated into the module was a micromanipulator for electrode placement. A Peltier thermoelectric unit, used in a feedback loop with a thermistor, maintained the axon temperature to within 0.2 °C of a fixed, adjustable value (12.5 or 15 °C). Axons were bathed in flowing filtered natural seawater or potassium-free artificial seawater (430 mM NaCl, 50 mM $MgCl_2$, 10 mM $CaCl_2$, 10 mM Tris) at a *p*H of about 7.8. Axons were exposed to helium pressures of up to 204 atm, pressure being applied at a rate of about 7 atm min^{-1}. The voltage-clamp design followed that of Armstrong *et al.*[9]. We used "piggyback" axial electrodes internally and platinised silver electrodes with guard regions externally. A glass pipette filled with 0.5 M KCl was used for external voltage reference.

After space-clamped action potentials had been obtained, the axon was voltage clamped with holding potential set at the initial resting potential of the axon. A family of current–time curves was obtained by means of depolarising square-wave pulses. Each of these pulses was preceded by a 10-ms, 40-mV hyperpolarising pulse in order to activate sodium channels fully. Leakage currents were derived by linear extrapolation of currents resulting from hyperpolarising pulses, and were subtracted from the observed clamp currents to obtain sodium and potassium currents. To calculate ionic conductances, these ionic currents were then divided by the difference between respective clamped membrane potentials and the observed ionic reversal potentials. The reversal potential for sodium was the clamped potential at which no sodium currents were seen; that for potassium was estimated in the unclamped axon from the maximum value of the after potential.

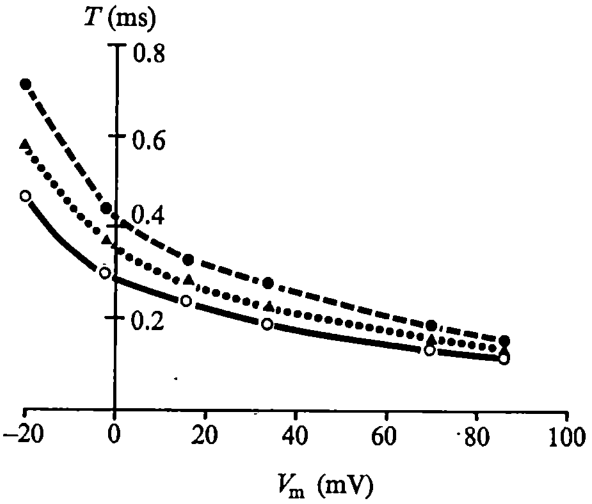

Fig. 1 Time-to-peak early current (T) plotted as a function of membrane voltage during a voltage-clamp pulse. Data were obtained at 1 atm (○), 158 atm He (●) and 79 atm He (▲), in that order. The temperature was 12.5 °C.

Effects of pressure were studied in nine axons. Action potentials obtained in the unclamped squid axon with helium pressure are comparable with those reported by Spyropoulos using hydrostatic pressure[4]; the most striking effect was a prolongation of the action potential. The amplitude of the action potential was either unaffected by helium pressure or decreased by about 10%. No significant change in resting membrane potential was observed.

In the voltage-clamped axon, pressure prolonged the time course of both early and late currents. These effects were entirely reversible and varied with pressure, as shown in Fig. 1. Here the time-to-peak early current is plotted as a function of clamped membrane potential. Data were obtained at 1 atm, 158 atm and 79 atm, in that order. Each time to peak current was increased by pressure, and lowering the pressure from 158 to 79 atm partially restored each time toward its control value. Figure 2 shows sodium and potassium currents plotted as functions of clamped membrane potential. In this experiment a reduction in the maximum inward sodium current occurred immediately upon reaching 204 atm, with little effect on potassium currents. In two axons, non-reversible decreases in sodium reversal potential of less than 10 mV were obtained. Lastly, little or no change in the calculated maximum sodium and potassium conductances occurred. The average maximum conductances for the nine axons expressed as a percentage of control were 93±2.9% for sodium and 91±4.2% for potassium. The small decrease of the maximum conductances probably reflects axon deterioration in some of the preparations.

Our results with voltage-clamped squid axon show that pressure slows the time course of both early and late ionic currents. Since in most cases the maximum conductances for sodium and potassium were not significantly affected by pressure, it is likely that the number of ionic channels which open during excitation does not change and that pressure affects the kinetics of channel opening and closing; that is, the "gating" processes are primarily affected.

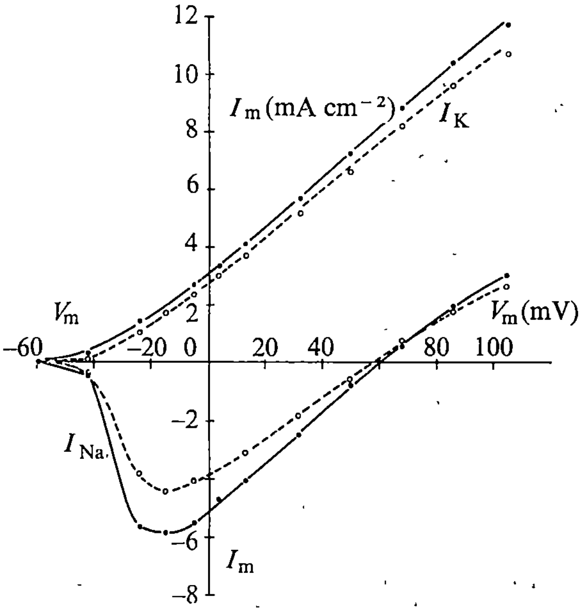

Fig. 2 Current–voltage relationship for sodium current (I_{Na}) and potassium current (I_K) at 1 atm (●) and 204 atm He (○). The temperature was 15 °C.

Further, the reduction of sodium current amplitude shown in Fig. 2 is explainable in terms of altered rate processes if we assume that gating for sodium activation is slowed but sodium inactivation is not affected by pressure. We were unable to study this behaviour after reducing pressure since sodium currents decreased further with decompression. Potassium current amplitudes also decreased by about 20% with decompression; however, we feel that these later changes are brought about by damage caused by the formation of helium gas bubbles during decompression.

To test our conclusions we carried out computer simulation, using the HH equations[7] to generate voltage-clamp curves[10] and action potentials (modified from ref. 11). By reducing the αs and βs for the sodium activation (m) and potassium activation (n) processes, but not the sodium inactivation (h) process, by about 30%, all our experimental curves were well approximated. Other combinations were tested, including alteration of the h process, but the curves obtained were not consistent with our data.

The effect of pressure on rate processes is predicted by the relation[5]

$$k_P = k_0 \exp(-P\Delta V^{\ddagger}/RT)$$

where k_P is a rate constant at pressure P, k_0 is the rate constant at 1 atm, $\Delta V^{\ddagger}$ is the volume of activation, that is, the volume change of reactants in going to the activated state, R is the gas constant and T is the absolute temperature. Assuming that the αs and βs of the HH equations represent rate coefficients for gating reactions in ionic channels, the above expression can be used to estimate the volumes of activation for the m and n processes. Although subject to large errors, these estimates, ranging from 30 to 75 cm^3 mol^{-1}, lie in the range of many macromolecular reactions[5].

A practical consideration in this study is the recently described high pressure neurological syndrome[12]. Human divers and many animals subjected to helium[13,14] or hydrostatic[14,15] pressures develop coarse tremor, loss of sense of limb position, incoordination and disturbances of consciousness[15]. Although the effects on channel kinetics that we observed may be related to this syndrome, the connection is so far ambiguous since synapses are also likely to be affected by pressure[16]. Nevertheless, the voltage-clamp data form a basis for discussion at the molecular level of the neurophysiological effects of pressure.

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Role of PEP carboxylase during seed development in *Pisum sativum*

Pisum sativum has been put forward as a practical alternative to soybean (*Glycine max*) as a protein source that can be grown in temperate climates. In contrast to the cereals little attention has been paid to the contribution of the reproductive parts of *Pisum sativum* in the carbon economy of the developing seed. This information would be important in subsequent breeding programmes designed to exploit knowledge of the physiological components of yield.

It has been thought that the pod wall of *P. sativum* could refix, by photosynthesis, respired CO_2 from the developing seeds and then recycle it to the seeds[1]. Although assimilates are obviously transferred from the pod wall to the seed, the refixation hypothesis conflicts with the estimations of pea pod photosynthesis. Pods of *P. sativum* have very low rates of photosynthesis relative to the leaves[2], as have those of other leguminous plants such as *G. max*[3,4] and French bean (*Phaseolus vulgaris*)[5]. The function of the pod as a photosynthetic organ is therefore uncertain since the amount of CO_2 which could be fixed would be minimal compared with the large amount of CO_2 respired from the seed, and even this minimal fixation could only occur during the day. A more satisfactory way of reducing respiratory losses of CO_2 would be through a dark fixation system, which would work during the night and could also exist within the pea seed, which develops in perpetual low light intensity. Dark fixation systems have been described for the leaves of C3 species, for example, tobacco[6] and barley[7]. In tobacco the initial products of this dark fixation are similar to those found in *Bryophyllum calcinum*, which has the crassulacean acid metabolism (CAM) type of photosynthetic system, depending on dark fixation of CO_2 through phosphoenol pyruvate (PEP) carboxylase. Therefore we have looked for this enzyme in extracts from developing pod walls, testae and tissue consisting of cotyledon plus embryonic axis. The activity of the photosynthetic CO_2 fixation enzyme RuDP carboxylase, was also determined using the same extracts. Isocitrate dehydrogenase (NADP-dependent) activity was assayed as this enzyme is associated with mitochondrial respiration.

PEP carboxylase activity was found in all the tissues sampled (Fig. 1). The developmental patterns of activity for this enzyme in extracts from pod walls and testae were similar, with the highest level of activity found when the tissues were youngest, the activity then decreasing as ageing proceeded. The specific activities for PEP carboxylase within the two tissues, however, were different. The maximum PEP carboxylase activity found in the pod wall was relatively low and similar to that found in the young leaves of pea, (C.L.H., unpublished) and young leaves of *Antirrhinum majus*[8]. Much greater specific activities, however, were found in the testa, the highest enzyme levels being equivalent to the PEP carboxylase activity extracted from maize plants using a similar assay and extraction procedure (unpublished data of C.L.H.). The pattern of PEP carboxylase activity for cotyledons was opposite to that found during the development of pod walls and testae. The lowest level of activity was found when the cotyledons were young and the activity increased throughout development.

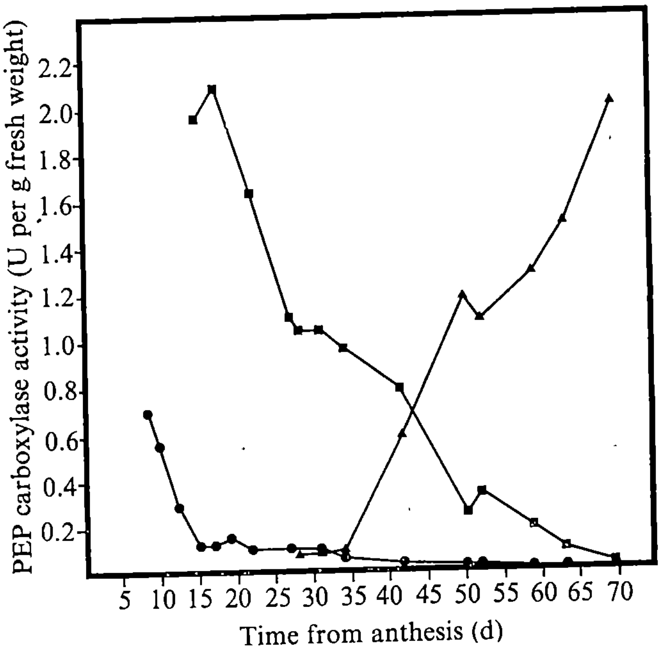

Fig. 1 Changes in the activity of PEP carboxylase extracted from pod wall (●), testa (■) and cotyledon (▲) tissue of *P. sativum*. The activity of PEP carboxylase was estimated by following the conversion of NADH to NAD in the presence of phosphoenol pyruvate at 340 nm wavelength[13]. The enzyme was coupled with malic dehydrogenase. Total reaction volume of 3.0 ml included the following: 30 μM bicine; 30 μM $MgCl_2$; 0.5 μM dithiothreitol; 4.2 U of malic dehydrogenase, and 0.25 μM NADH. The reaction was started by the addition of 3 μM phosphoenol pyruvate. The reaction mixture was buffered to *p*H 7.8 and the temperature was maintained at 25 °C. Enzyme activity was calculated using the extinction coefficient of NADH (6.22×10^6 cm^2 mol^{-1}). The enzyme extract was prepared by homogenising 200 mg of tissue in 2 ml of buffer composed of 0.04 M Tris-(hydroxymethyl)-methylamine; 0.01 M $MgCl_2$ and 0.5 mM dithiothreitol, and adjusted to *p*H 7.5. The supernatant obtained by 20 min centrifugation at 40,000*g* was used as the enzyme source.

Table 1 Changes in RuDP carboxylase and isocitrate dehydrogenase during the development of the pod wall, testa and cotyledons

Days from anthesis	RuDP-carboxylase (mU per g fresh weight) Pod wall	Testa	Cotyledons	Isocitrate dehydrogenase (mU per g fresh weight) Pod wall	Testa	Cotyledons
8	40	—	—	1,900	—	—
10	80	—	—	1,300	—	—
15	40	20	—	1,080	3,040	—
18	30	10	—	1,070	4,280	—
22	40	0	—	1,080	3,050	—
28	40	0	0	1,300	3,050	960
34	5	10	0	1,350	3,400	1,120
42	0	10	0	880	2,530	1,230
50	0	0	0	680	1,910	1,210
59	0	0	20	410	1,500	660
69	0	0	40	100	350	700
Activity of young fully expanded leaf		3,320			1,620	

Enzyme extracts were prepared as described in Fig. 1. RuDP carboxylase activity was estimated by following the conversion of NADH to NAD in the presence of ribulose-1,5-diphosphate at 340 nm wavelength[12,13]. The total reaction volume of 3.0 ml included: 30 μM bicine; 30 μM $MgCl_2$; 0.5 μM dithiothreitol; 150 μM $NaHCO_3$; 10 μM ATP; 0 5 μM NADH; 4 U of glyceraldehyde-3-phosphate dehydrogenase, and 12 5 U 3-phosphoglycerate kinase. The reaction mixture was buffered to *p*H 7.8 and the reaction temperature maintained at 25 °C. Enzyme activity was calculated using the extinction coefficient of NADH (6.22×10^6 cm^2 mol^{-1}). Isocitrate dehydrogenase activity was estimated by following the conversion of NADP to NADPH in the presence of isocitric acid at 340 nm wavelength[14]. The total reaction volume of 3.0 ml included the following: 250 μM Tris-(hydroxymethyl)-methylamine; 5 μM glutathione; 12 μM isocitric acid; 125 μM NaCl; 1 μM NADP and 12 μM $MnSO_4$. The reaction mixture was buffered to *p*H 7.8 and the temperature kept at 25 °C. Enzyme activity was calculated by using the extinction coefficient of NADPH (6.22×10^6 cm^2 mol^{-1}).

Table 2 Changes in net CO_2 uptake during the development of pea pods

Days from anthesis	Net CO_2 uptake in the light (60 klx) (mg CO_2 dm^{-2} h^{-1})	Net CO_2 uptake in the dark (mg CO_2 dm^{-2} h^{-1})
8	−1.25	−12.01
12	+1.75	−4.52
14	+2.44	−4.13
19	+1.07	−3.72
21	+0.63	−4.63
25	−0.63	−4.32
33	−1.21	−4.64
39	−0.87	−5.90
47	−1.38	−5.03
Net CO_2 uptake of young fully expanded leaf	+31.90	−0.88

Net CO_2 uptake was measured using a multichannel infrared gas analyser system in which CO_2 concentration, light intensity and temperature could be varied independently[15]. Net CO_2 uptake is represented as positive and net CO_2 output as negative values. Each value in the table is the mean derived from the CO_2 exchange of at least six pods.

Isocitrate dehydrogenase activity was high during the early part of development of the three tissues (Table 1) and then decreased to reach a low level at the time of seed desiccation. The maximum specific activity found in the testae was approximately three times the activity found in either the pod wall, the cotyledons or young fully expanded leaves.

The activity of RuDP carboxylase in the pod wall (Table 1) was from 10 to 100 times less than the activity found in leaves and it was difficult to detect any activity for this enzyme in extracts of testae or cotyledons. The low level of RuDP carboxylase activity extracted from pod walls correlated with the low rates of net CO_2 uptake (Table 2), a net uptake of CO_2 being shown only during the first 21 d of pod development after anthesis.

The relatively high PEP carboxylase activity found in the testae and developing cotyledons suggests that recycling of respired CO_2 could occur at the site of origin within the seed. The positive correlation which exists, however, between PEP carboxylase and isocitrate dehydrogenase activity during the development of both the pod wall and the testa is not apparent for the cotyledons.

The main function of the PEP carboxylase synthesised in the cotyledons may therefore be in recycling CO_2 during germination, and not necessarily during the development of the seed. This suggestion is supported by the finding that the high PEP carboxylase activity in mature cotyledons is still present in dry seed and is maintained for the initial period of germination (C.L.H., unpublished). The level of PEP carboxylase in the pod wall declines before the development of the testae and cotyledons and it is unlikely therefore that the pod wall has an important role in fixing respired CO_2 from the developing seed.

There is increasing evidence therefore that PEP carboxylase, which is usually considered to be associated with C4 and CAM photosynthetic systems, is also involved in the development of plants with the C3 type of photosynthesis. In C3 plants PEP carboxylase activity seems to be highest during developmental phases when the rate of respiration exceeds the rate of photosynthesis. Much of the evidence supporting this hypothesis has come from developmental studies of the leaves of tobacco[9,10], citrus[10] and *Antirrhinum majus*[8]. These and our observations may have implications regarding the evolution of the C4 photosynthetic system. It can be suggested that high levels of PEP carboxylase are induced when there is a net loss of CO_2. Plants which have evolved in temperate conditions would exhibit such a net carbon loss only a few specific times in development, or in particular specialised parts of the plant, such as the developing seed. Plant species, however, which have evolved in environments which would normally induce high rates of mitochondrial respiration and photorespiration, would have a selective advantage in maintaining a system for refixing respired CO_2 for longer periods during development. C4 plants may have evolved initially by selection of variants in which the trigger for cessation of PEP carboxylase synthesis operated later in development. A late switching from a C4 to a C3 system has been suggested for leaves of the C4 plant *Portulaca oleracea*[11].

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Inactive complex formation between *E. coli* RNA polymerase and inhibitor protein purified from T7 phage infected cells

The "host shut-off" function of T7 phage has been thought to result from an inhibition of the host *Escherichia coli* RNA polymerase, which transcribes host RNA and T7 early mRNA, by an early T7 protein presumably the product of gene 0.7, or the "host shut-off" gene[1–4]. When T7 infection proceeds, early mRNA synthesis is shut off and the T7-specific RNA polymerase[5], the product of an early gene of T7 (gene 1), transcribes late mRNA. Thus, "host shut-off" is another control factor involved in the early–late switch of T7 gene expression in addition to the switch of two RNA polymerases from the host to the phage-coded enzyme transcribing early mRNA and late mRNA, respectively, at different times of infection[3].

We reported previously[6] that the "host shut-off" function of T7 is indeed an inactivation of the host RNA polymerase by an inhibitor protein bound to the enzyme. We found that when this inhibitor protein is removed from the inactive host RNA polymerase prepared from T7 infected cells, the core enzyme and the sigma factor are both functionally active[6]. The inhibitor protein did not inhibit T7-specific RNA polymerase or the *E. coli* RNA polymerase core enzyme[6].

We report here further characterisations of the inhibitor protein (I protein). I protein was purified to an electrophoretic homogeneity, with a molecular weight of approximately 14,000, by Sephadex G-100 column chromatography. The inhibition of *E. coli* RNA polymerase holoenzyme by I protein was shown to result from a direct binding of I protein to the enzyme, thus blocking the enzyme from binding to template DNA and initiating RNA synthesis.

In our previous experiments[6], we used a procedure similar to that described by Burgess[7] to partially purify *E. coli* RNA polymerase up to the first glycerol gradient (without KCl) centrifugation step. The enzyme obtained from T7 infected cells was only 5% as active as the enzyme obtained from uninfected cells due to the association of the inhibitor to the enzyme. I protein was separated from the RNA polymerase by phosphocellulose column chromatography, and then purified by DEAE cellulose chromatography followed by glycerol gradient centrifugation. The inhibitor material after the glycerol gradient step was subjected to acrylamide–sodium dodecyl sulphate (SDS) gel electrophoresis and showed one major stainable protein with a molecular weight of about 66,000 (ref. 6)

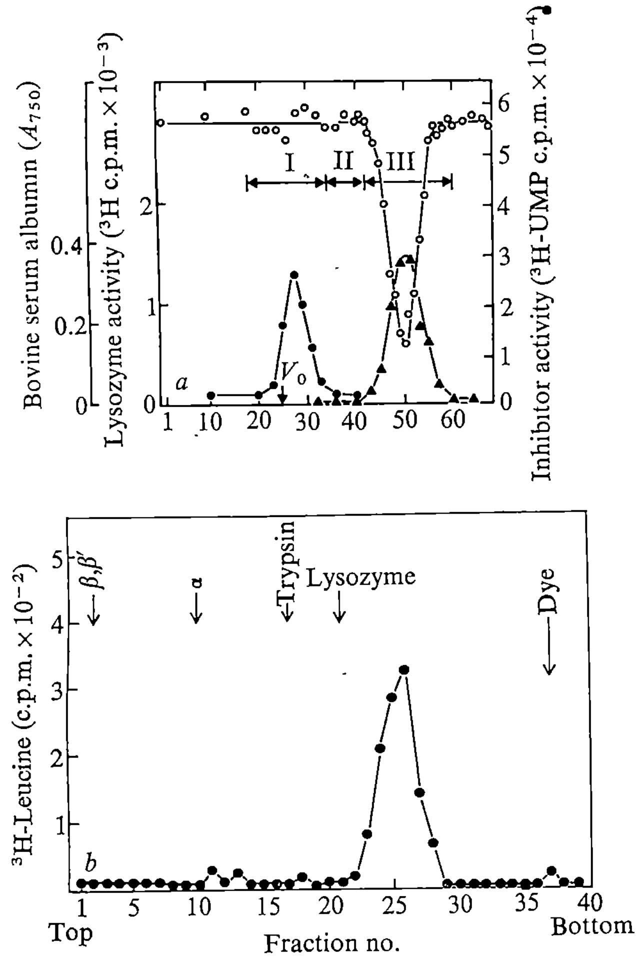


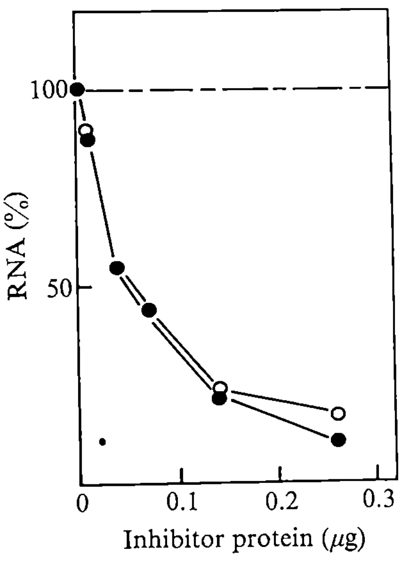
Fig. 1 *a*, Purification of I protein by Sephadex G-100 column chromatography. Sephadex G-100 column (1.2×45 cm) was calibrated using the following standards: bovine serum albumin (molecular weight 68,000), ovalbumin (45,000), trypsin (24,500) and lysozyme (14,500). Partially purified inhibitor protein (after the phosphocellulose and DEAE chromatography step[6]) prepared from T7 infected *E. coli* D10 F$^-$ cells (labelled with ^{3}H-leucine (1 mCi per 8 μg per ml) for two generations before infection and with ^{35}S-methionine (950 μCi μg^{-1} ml^{-1}) for 9 min after infection) was applied to the Sephadex column and eluted with a 0.01 M Tris-HCl buffer, *p*H 7.9, containing 0.05 M KCl, 0.01 M MgCl$_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA and 2% glycerol. Each fraction was assayed for the inhibitory activity on *E. coli* RNA polymerase (reduction in RNA synthesis measured by ^{3}H-UMP incorporation in the standard assay conditions with T7 DNA[6]). Fractions designated as I, II and III were pooled, concentrated and analysed by electrophoresis (see *b*). ○, Inhibitor activity, reduction in c.p.m. ^{3}H-UMP incorporated into RNA. ●, Bovine serum albumin, A_{750} value after the reaction of Lowry *et al.*[14]. ▲, Lysozyme, activity in c.p.m. ^{3}H-diaminopimelic acid assayed by the method of Schweiger and Gold[15] *b*, Acrylamide–SDS gel electrophoretic profile of purified I protein. I protein was purified as described in *a* from T7 infected cells labelled with ^{3}H-leucine (5 mCi per 2.5 μg per ml) for 9 min after infection Pool III material of Sephadex G-100 column fractions, similar to that shown in *a*, was subjected to acrylamide (15%)–SDS slab gel electrophoresis[16], and the radioactivity of ^{3}H-leucine in each gel slice was counted. Marker proteins, *E. coli* RNA polymerase β′, β and α subunits, trypsin and lysozyme, were electrophoressed in the same slab gel.

We found, however, that the protein which is the true inhibitor of *E. coil* RNA polymerase (the I protein) has a molecular weight of approximately 14,000 by further purifying the inhibitor material. In this experiment, a cell-free extract prepared from T7 infected cells that had been labelled with ^{3}H-leucine before infection and with ^{35}S-methionine for 9 min after infection was used to purify the I protein Purification was accomplished by the use of Sephadex G-100 column chromatography instead of previously used glycerol gradient centrifugation after phosphocellulose and DEAE cellulose chromatography of the inhibitor material.

As shown in Fig. 1*a*, the activity of I protein (extent of inhibition on *E coli* RNA polymerase) was eluted in the Sephadex elution fractions designated as pool III in Fig 1, and the molecular weight of I protein was estimated to be about 14,000 from the molecular weights of reference proteins. No inhibitory activity on *E. coli* RNA polymerase was found in the elution fractions designated as pool I in Fig. 1 which contained a marker protein bovine serum albumin with a molecular weight of 68,000. Materials in pools I and III shown in Fig. 1*a* were concentrated and subjected to acrylamide–SDS gel electrophoresis and the radioactivity of each gel slice was measured. Pool I which had no inhibitor activity yielded a protein labelled mostly with ^{3}H-leucine and a molecular weight about 66,000 as the major component. Pool III showed only one protein exclusively labelled with ^{35}S-methionine.

Figure 1*b* shows the electrophoretic profile in acrylamide–SDS gel of I protein purified similarly from T7 infected cells which were labelled with ^{3}H-leucine for 9 min only after T7 infection. ^{3}H-leucine-labelled I protein was the sole component of the Sephadex elution fractions which contained the inhibitor activity (same as the result in Fig. 1*a*, pool III) and this protein migrated in the gel faster than the lysozyme marker (molecular weight 14,500) Glycerol gradient centrifugation of purified I protein, labelled with ^{35}S-methionine or ^{3}H-leucine, also gave an estimated

Fig. 2 Inhibition of *E. coli* RNA polymerase holoenzyme by purified I protein. Standard RNA-synthesising system[6] contained ^{3}H-UTP (1 μCi per 50 nmol), 5 μg T7 DNA, or T4 DNA, or poly d(AT), and 0.85 μg purified *E. coli* RNA polymerase holoenzyme saturated with purified sigma factor[17] in 0.25 ml. Purified I protein was added as indicated. ^{3}H-UMP incorporated into RNA after 10 min at 37 °C was measured. ○ Poly d(AT)-directed RNA synthesis. The 100% value was 65,557 c.p.m. ●, T7 DNA-directed RNA synthesis. The 100% value was 47,351 c.p.m T4 DNA-directed RNA synthesis was similar, and not shown. -----, Effect of I protein on RNA synthesis by T7-specific RNA polymerase on T7 DNA and by *E. coli* RNA polymerase core enzyme on poly d(AT) assayed as described previously[6].



molecular weight of about 14,000 for I protein (data not shown). Therefore, we conclude that the I protein is a protein synthesised only after T7 infection and has a molecular weight of about 14,000.

Purified I protein inhibited the synthesis of RNA by *E. coli* RNA polymerase holoenzyme on T4 DNA, T7 DNA and poly d(AT) as shown in Fig 2. At 50% inhibition, the molar ratio of I protein to the enzyme was about 1 : 1.5 (0.04 μg I protein : 0.85 μg holoenzyme, using molecular weights 14,000 and 500,000, respectively). On the other hand, as described previously[6], I protein showed no inhibition on the synthesis of RNA by T7-specific polymerase on T7 DNA or *E. coli* RNA polymerase core enzyme on poly d(AT).

Figure 3 shows that the inhibition of *E. coli* RNA polymerase holoenzyme by I protein is due to a direct binding of I protein to the enzyme in the absence of template DNA. Purified I protein labelled with ^{3}H-leucine formed a tight complex with the enzyme, which was not dissociated by gradient centrifugation (Fig. 3*b*), with a concomitant loss in the activity of the RNA polymerase (Fig. 3*a*) and the inhibitory activity of I protein (Fig. 3*c*). I protein did not form a complex with the sigma factor when tested in a similar glycerol gradient centrifugation (data not shown).

Inhibition of *E. coli* RNA polymerase holoenzyme by I protein is limited to the initiation of RNA synthesis by the enzyme. Figure 4 shows that the effect of I protein (Fig. 4*b*) is almost identical to that of rifampicin (Fig. 4*a*) on the T7 DNA-directed RNA synthesis. Addition of I protein to the already initiated RNA-synthesising system did not stop the RNA synthesis immediately and allowed a residual synthesis of RNA, supposedly a completion of one round of RNA synthesis already initiated by the enzyme. If the I protein was added to the enzyme before the template DNA, the reaction was completely inhibited. If the enzyme and T7 DNA were, however, mixed before the addition of I protein and the substrates nucleoside triphosphates, one round of RNA synthesis took place.

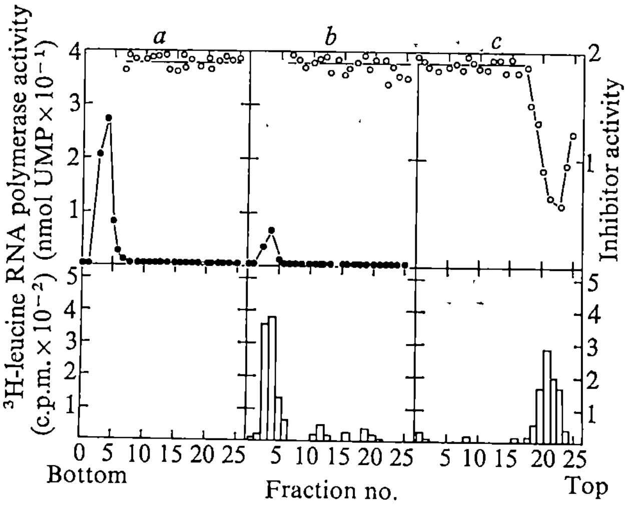


Fig. 3 Binding of ^{3}H-leucine-labelled I protein to *E. coli* RNA polymerase analysed by glycerol gradient centrifugation. Three samples contained: *a*, *E coli* RNA polymerase holoenzyme (62 µg); *b*, *E. coli* RNA polymerase holoenzyme (62 µg) and purified I protein labelled with ^{3}H-leucine (968 c.p.m., the material shown in Fig. 1*b*), and *c*, ^{3}H-leucine-labelled I protein (968 c.p.m.). These samples were centrifuged in 12 ml of 10–30% glycerol gradients in 0.02 M Tris-HCl, *p*H 7.9, 0.15 M KCl, 0.01 M MgCl$_2$, 0.1 mM dithiothreitol and 0.1 mM EDTA at 40,000 r.p.m. for 20 h using an SW41 rotor. Each gradient fraction (10 µl) from *a* and *b* was assayed for the *E. coli* RNA polymerase activity in a standard system[6] with 10 µg T7 DNA, and the activity was expressed as nmol ^{3}H-UMP incorporated into RNA. Each gradient fraction (30 µl) from all three gradients was assayed for the inhibitor activity in a reaction mixture containing 10 µg T7 DNA and 3 µg purified *E. coli* RNA polymerase holoenzyme and plotted as a reduction in nmol ^{3}H-UMP incorporated into RNA due to the inhibitor. The rest of each fraction from *b* and *c* was used for the measurement of acid-insoluble radioactivity of ^{3}H-leucine after the addition of 200 µg bovine serum albumin. ●, RNA polymerase activity, nmol UMP incorporated into RNA. ○, Inhibitor activity, reduction in nmol UMP incorporated into RNA in T7 DNA-directed RNA-synthesising system Open colums, Radioactivity of ^{3}H-leucine-labelled I protein.

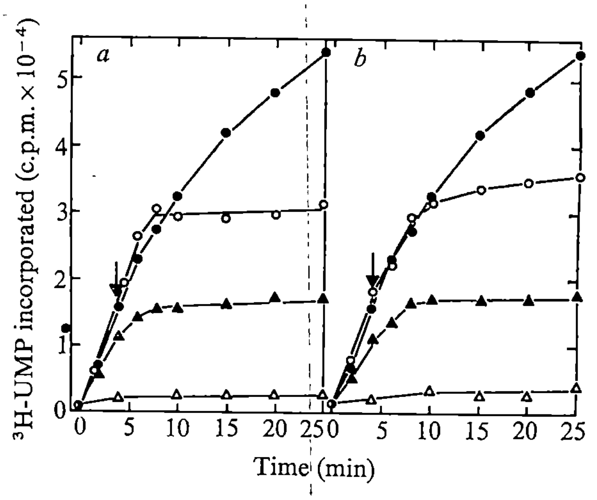


Fig. 4 Inhibition of T7 DNA-directed RNA synthesis at initiation by rifampicin (*a*) and I protein (*b*). T7 DNA-directed RNA synthesis was carried out using the standard system[6] containing 12 µg T7 DNA and 2 µg purified *E coli* RNA polymerase holoenzyme in a total volume of 2.5 ml at 37 °C. From each reaction mixture, 0.25 ml aliquots were withdrawn at indicated times and ^{3}H-UMP incorporated into acid-insoluble material from ^{3}H-UTP was measured. In *a*, 2 µg per 0.25 ml rifampicin was added as indicated. In *b*, approximately 0.15 µg per 0.25 ml of purified I protein was added when indicated. ●, Control, no rifampicin or I protein. ○, Normal reaction was started at 0 time. Rifampicin or I protein was added at 4 minutes (indicated by an arrow). △, RNA polymerase was incubated with rifampicin or I protein at 37 °C for 30 s, and then T7 DNA, ^{3}H-UTP and other nucleoside triphosphates were added at 0 time. ▲, RNA polymerase and T7 DNA were incubated at 37 °C for 5 min, and then rifampicin or I protein was added together with ^{3}H-UTP and other nucleoside triphosphates at 0 time.

The direct binding of I protein to the RNA polymerase described above (Fig. 3) explains the results shown in Fig. 4. A complex formed between the enzyme and I protein is inactive to initiate RNA synthesis. In addition, we found that the binding of I protein to the RNA polymerase blocks the enzyme to bind to the template DNA, as will be published elsewhere. Radioactive T7 DNA can be retained on Millipore filters if the DNA is incubated with *E. coli* RNA polymerase before filtration as described by Hinkle and Chamberlin[8]. When the RNA polymerase and I protein were incubated and then interacted with T7 DNA, DNA retention on the filter was greatly reduced.

After our previous publication[6], Ponta *et al.*[9] reported an isolation of *E. coli* RNA polymerase inhibitor from T7 infected cells. This protein, with an estimated molecular weight of about 14,000, inhibits the RNA synthesis by the *E. coli* RNA polymerase holoenzyme at initiation but has no effect on the T7-specific RNA polymerase and only partially inhibits the core enzyme of *E. coli*. It seems that the inhibitor protein reported by Ponta *et al.* is similar or identical to the I protein described here. Whereas Ponta *et al.* suggested that the inhibitor protein interacts with the RNA polymerase or the RNA polymerase–DNA complex[9], we have presented evidence here that the I protein binds to the RNA polymerase in the absence of DNA and forms an inactive complex which cannot initiate RNA synthesis.

As indicated previously[6], I protein is probably a T7 early protein. The fact that I protein is labelled exclusively by a radioactive amino acid added only after T7 infection (results in Fig. 1) supports this notion. Although T7 gene

0.7 is termed as the "host shut-off" gene[3-4], cell-free extracts from the cells infected with T7 mutant phages with mutations in gene 0.7 (H3 and A57, refs 10 and 11) showed a complete inactivation of the *E. coli* RNA polymerase, that is, the "host shut-off" function was operating in these mutant phage-infected cells. At present, T7 gene 0.3 is the most likely candidate to code for the I protein, since Ratner[12] has shown that the gene 0.3 protein, with a molecular weight of 14,000, is the only T7 protein which binds to *E. coli* RNA polymerase. Although gene 0.3 has been regarded as a non-essential gene of T7 (refs 3, 10 and 11), it is interesting to note that Studier[13] has found the gene 0.3 protein as an anti-host restriction protein. It is conceivable that a protein coded by a small phage, such as gene 0.3 protein of T7, possesses dual functions

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Yeast mitochondrial RNA contains a short polyadenylic acid segment

POLYADENYLIC acid (poly(A)) is present at the 3′ terminus of most classes of cytoplasmic messenger RNAs (mRNAs) in all eukaryotic organisms reported so far[1,2]. Although the role of poly(A) in mRNA function and metabolism is still a subject for speculation[1], its presence has provided a convenient means for isolation of this class of mRNA by its ability to bind to cellulose and nitrocellulose filters, and to hybridise with matrices containing either oligo(dT)[3] or poly(U)[4]. Most poly(A) in cytoplasmic mRNA has been found to be 100–150 nucleotides long, with an electrophoretic mobility of about 7S (ref. 1). The poly(A) segment length of yeast cytoplasmic mRNA is only 50–60 nucleotides, with an electrophoretic mobility of about 4S[5]. mRNA species with still shorter poly(A) segments of only 19–34 residues have been described in the silk moth[6].

Mitochondrial mRNA of higher organisms also contains poly(A) at its 3′ terminus. HeLa cell mitochondrial mRNA has a poly(A) segment of about 50–70 nucleotides, and presumptive mRNAs that bind to oligo(dT) have been found in mitochondria of several higher animals[7]. Therefore, the report of Groot *et al.*[8] that they were unable to detect poly(A)-containing RNA in mitochondria of *Saccharomyces carlsbergensis* was interesting. Not only did this suggest that the relatively simple affinity chromotographic procedures using oligo(dT) or poly(U) were not applicable to the isolation of yeast mitochondrial mRNA, but it also apparently represented another example of the similarity between mitochondria and prokaryotes. These workers suggested that addition of poly(A) to mitochondrial mRNA of higher eukaryotes was an adaptation in response to the loss of approximately 80% of the potential information in the mitochondrial genome of animals compared with yeast. Most recently, however, prokaryote RNA also has been shown to contain poly(A) segments[9,10].

Our laboratory has been interested in the isolation, characterisation, and translation of mRNA from yeast mitochondria. In contrast to the findings of Groot *et al.*[8], we have been able to isolate poly(A)-containing RNA from *Saccharomyces cerevisiae* using poly(U) Sepharose chromatography. This RNA contains a short poly(A) segment of approximately 20–30 nucleotides. The small size of the poly(A) segment may account for the failure of Groot *et al.* to detect yeast mitochondrial poly(A)-containing RNA.

We used the haploid strain of *Saccharomyces cerevisiae,* D243-2B-R1 (ref. 11). Cells were grown to a mid-exponential phase and were collected as described by Casey *et al*[11]. Protoplasts and mitochondria were prepared according to the method of Grivell and Borst[12], except that preincubation of yeast before exposure to snail digestive enzyme (Glusulase, Endo Inc., Garden City, New York) was omitted, and protoplasts were washed only once with 1.5 M sorbitol buffer before homogenisation. The mitochondria were lysed in 100 mM NaCl, 2 mM EDTA, 10 mM Tris (*p*H 7.8), 0.2% sodium dodecyl sulphate containing polyvinyl sulphate (20 μg ml^{-1}) and heparin (200 μg ml^{-1}). Mitochondrial RNA was isolated from lysed mitochondria by the phenol/chloroform–isoamyl alcohol extraction procedure[13].

A definitive fraction of the ^{32}P- and ^{3}H-adenine steady-state labelled RNA isolated from yeast mitochondria is retained on the poly(U) Sepharose column under high salt conditions and is eluted with 90% formamide (Table 1). This material (fraction II) accounts for about 2.0% of the ^{32}P label, 6.1% of the ^{3}H-adenine, and 5.0% of the A_{260} of the total mitochondrial RNA preparation. The greater

Table 1 Fractionation of yeast mitochondrial RNA by poly(U) Sepharose 4B chromatography

RNA fraction	Recovery		
	A_{260}	^{32}P (c.p.m. × 10^6)	^{3}H (c.p.m. × 10^6)
Total RNA	250.6	128	46
Fraction II (poly(A)-containing RNA)			
First purification	45.9	10.1	9.6
Second purification	14.1	2.9	3.1
Third purification	12.6	2.6	2.8
Poly(A) segment	—	0.019	0.058

Steady-state labelling of mitochondrial RNA was achieved by growing yeast with ^{32}P (orthophosphate, carrier-free, Schwarz-Mann) and 2-^{3}H-adenine (38 Ci mmol^{-1}, New England Nuclear Corp.) to the mid-exponential phase. Mitochondrial RNA was isolated as described in the text. Poly(A)-containing RNA was isolated with poly(U) Sepharose 4B by combination of the methods of Firtel *et al*[17] and Lindberg *et al.*[18]. Poly(U) Sepharose was equilibrated with high salt buffer (400 mM NaCl, 2 mM EDTA, 10 mM Tris, (*p*H 7.5) and 0.2% sodium dodecyl sulphate). Approximately 50 A_{260} U ml^{-1} of the mitochondrial RNA preparation in high salt buffer was applied per ml of column volume at 25 °C. After 10 min, the column was washed with 9 column volumes of high salt buffer. The first four volumes of wash were pooled, and the RNA was precipitated with 2.5 volumes of ethanol (fraction I). The poly(A)-containing RNA (fraction II) was eluted with 3 volumes of formamide buffer (90% formamide, 2 mM EDTA, and 10 mM potassium phosphate, *p*H 7.5). The RNA was precipitated with ethanol and subjected to two further cycles of poly(U) Sepharose chromatography. Poly(A) segments were isolated from poly(A)-containing RNA after pancreatic (Worthington) and T_1 RNase (Worthington) digestion by the method of Hirsch and Penman[13], followed by two cycles of poly(U) Sepharose chromatography.

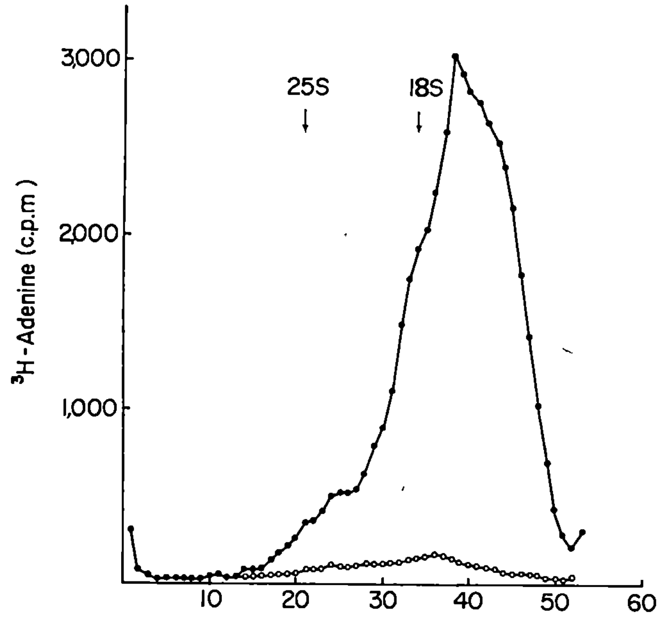

Fig. 1 5–20% sucrose gradient centrifugation of pulse-labelled yeast mitochondrial poly(A)-containing RNA. Protoplasts obtained from yeast grown to a mid-exponential phase were incubated for 15 min at 28 °C in 1.5 M sorbitol and 2% glucose containing ^{3}H-adenine (20 μCi ml^{-1}). Protoplasts were washed with carrier adenine and processed as described in the text and in the legend to Table 1. The poly(A) was isolated from these protoplasts (●), and from protoplasts which had been exposed to ethidium bromide (10 μg ml^{-1}) for 10 min before incubation with ^{3}H-adenine (○). The RNA was layered on 5–20% linear sucrose gradient and centrifuged in an SW 25.3 rotor at 24,000 r.p.m. for 20 h. Tubes were punctured from the bottom. Fractions were counted in Instagel (Packard).

yield of ^{3}H compared with ^{32}P in fraction II (subsequently referred to as poly(A)-containing RNA) can be explained only in part by the poly(A) content, and is probably due to the presence of labelled polyphosphates in the total mitochondrial RNA preparation. Approximately 2% of the ^{3}H-adenine in the poly(A)-containing RNA was recovered after T_1 and pancreatic RNase digestion followed by two additional cycles of poly(U) Sepharose chromatography (Table 1).

The isolated mitochondrial poly(A)-containing RNA had a high molecular weight, as indicated by sucrose gradient centrifugation (Fig. 1). The sucrose density gradient profile of the pulse-labelled poly(A)-containing RNA (Fig. 1) was heterogeneous, with a modal distribution of 16S which is similar to the pattern exhibited by HeLa cell mitochondrial poly(A)-containing RNA[13]. The absence of substantial contamination of poly(A)-containing RNA by cytoplasmic RNA is supported by the observation that ethidium bromide, a specific inhibitor of RNA transcription, inhibited ^{3}H-adenine incorporation by more than 90% (Fig. 1). Polyacrylamide gel electrophoretic analysis under denaturing and non-denaturing conditions reported elsewhere[14] also shows the poly(A)-containing RNA to be of high molecular weight, up to at least 23S, based on the electrophoretic mobility of mitochondrial ribosomal RNA. Hybridisation experiments indicate that there is little or no contamination with cytoplasmic poly(A)-containing RNA[14]. Specific activities of pulse-labelled poly(A)-containing RNA (fraction II) were 6–10 times greater than that of fraction I (that is RNA not retained by poly(U) Sepharose), which is consistent with a messenger role for poly(A)-containing RNA.

The electrophoretic behaviour in 12% acrylamide gels of the mitochondrial and cytoplasmic poly(A) segments isolated by RNase digestion of fraction II is shown in Fig. 2. The mitochondrial poly(A) segment migrates much more rapidly than cytoplasmic poly(A) and almost as rapidly as dA_{10} marker. Cytoplasmic poly(A) migrates slightly more rapidly than *E. coli* tRNA marker. The electrophoretic mobility of the mitochondrial poly(A) isolated from mechanically broken cells (Fig. 2) is similar to that obtained from protoplasts, but the cytoplasmic poly(A) segment isolated from protoplasts is more heterogeneous with a somewhat more rapid modal migration (data not shown). With both isolation procedures there was little (<5%) or no contamination of mitochondrial poly(A) with material having the mobility of cytoplasmic poly(A).

An estimate of the average number of adenylic acid residues in the poly(A) segment was also derived from the ratio of AMP to adenosine of alkaline hydrolysates of the segment (Table 2); values ranging from 20 to 28 nucleotides were obtained in four different mitochondrial RNA preparations. The presence of adenosine in alkaline hydrolysates of the poly(A) segment indicates that this segment is at the 3′ terminus of the poly(A)-containing RNA. The size of the segment seems to fall in the range of 20–30 nucleotides. The cytoplasmic poly(A) segment was about 50 nucleotides, which is consistent with that found by McLaughlin *et al.*[5].

Although these results provide strong evidence that a fraction of yeast mitochondrial RNA has a poly(A) segment at its 3′ end, it is conceivable that the poly(A) segment is internal and has been rendered terminal by the combined action of nucleases and phosphatase. We believe this possibility to be remote because of the high molecular weight of the poly(A)-containing RNA and the homogeneity of the poly(A) segment.

The inhibition of pulse labelling of the poly(A)-containing RNA by ethidium bromide, the distinct difference between the electrophoretic behaviour of the poly(A) segments from yeast mitochondrial RNA and that of the cytoplasmic poly(A) and the hybridisation data[14] indicate that the results are not due to cytoplasmic contamination. The size of the short poly(A) segment of yeast mitochondrial RNA is similar to that observed in *E coli*[9], *Dictyostelium discoideum*[15], and in silk moth chorion mRNA[6].

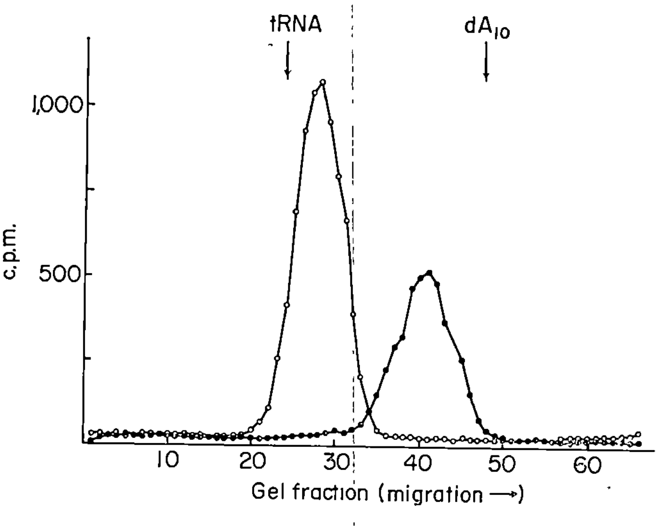

Fig. 2 Gel electrophoretic analysis of the poly(A) segment isolated from yeast cytoplasmic and mitochondrial poly(A)-containing RNA. The poly(A) segment was isolated as described in Table 1. Aliquots of 5,000 c.p.m. were electrophoresed on 12% acrylamide gels using Loening's E buffer[19] at 5 mA per gel for 1.75 h at 25 °C. Gels were sliced in a Savant Autogel divider and counted in 5 ml Instagel. Internal markers of dA_{10} and yeast tRNA were used and identified by absorbance at A_{260}. The length of the poly(A) segment was estimated by the migration relative to the standards. ●, ^{3}H-mitochondrial poly(A) segment; ○, ^{3}H-cytoplasmic poly(A) segment.

Table 2 Alkaline hydrolysis of the poly(A) segment

	Mitochondrial poly(A) (c.p.m)				Cytoplasmic poly(A) (c p.m.)
Preparation	1	2	3	4	4
AMP	2,200	8,725	2,063	3,641	5,870
Adenosine	96	391	93	179	115
AMP/adenosine	22.9	23.5	27.5	20 4	51.0

Four ^{3}H-poly(A)-containing RNA preparations, isolated as described in Table 1, were hydrolysed in 0.3 N KOH for 16 h at 37 °C. After neutralisation with $HClO_4$, paper electrophoresis was carried out in 50 mM sodium formate (*p*H 3.5), 2 mM EDTA at 4,000 V for 2.5 h in a Gilson Electrophoretor. Nucleotide and adenosine spots were eluted and counted, or the entire electrophorogram was cut into 1-cm strips and counted in Triton X-100 liquid scintillation fluid. Preparations 1–3 were isolated from spheroplasts whereas preparation 4 was isolated from mechanically broken cells.

That the mitochondrial poly(A)-containing RNA is mRNA is suggested by pulse label experiments. We have also demonstrated[14] that the poly(A)-containing RNA can direct, in an *E. coli* cell-free system, the translation of the three peptides of yeast cytochrome oxidase that have been shown to be synthesised *in vivo* by yeast mitochondrial ribosomes[16].

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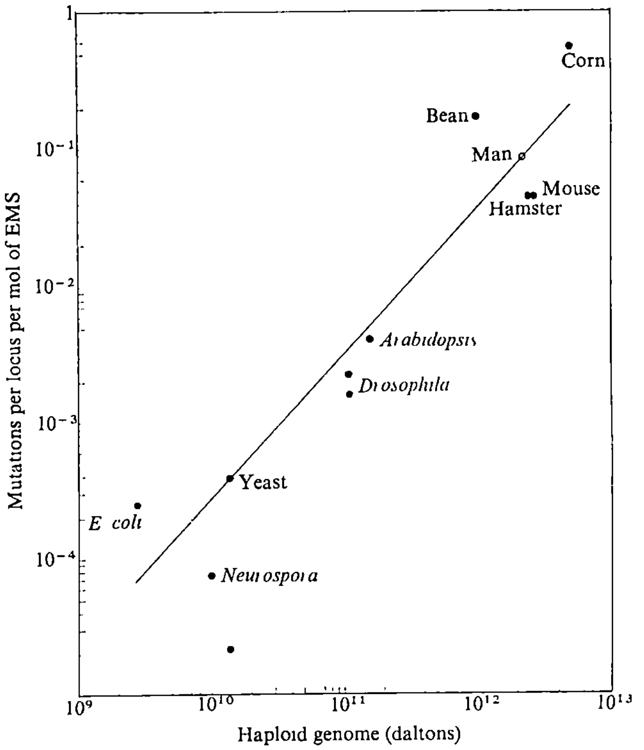
Mutation rate, genome size and their relation to the rec concept

ORGANISMS vary greatly in genome size, that is, in the amount of DNA comprising the haploid genome. Rates of radiation-induced mutation expressed as mutations per locus per rad, also differ widely among organisms, but it is surprising that mutation rates and genome size are correlated[1]. This correlation is unexpected because proteins are of a similar size in all organisms, implying that structural genes must also be of a similar size. Accordingly, one would expect that, although the overall mutation rate would be proportional to the overall number of genes, the mutation rate per locus would be similar in all organisms or, at least, bear no relation to genome size. Surveying published data, however, Abrahamson *et al.*[1] found a direct correlation between rate of radiation-induced mutation and size of the haploid genome from *Escherichia coli* to *Hordeum vulgare* (the ABCW relationship). We wondered whether rates of chemically induced mutation would also be related to genome size, both because of the practical importance of being able to extrapolate to the genetic effect of a chemical on the human population and because the information might help to elucidate the mechanism underlying the ABCW relationship.

We therefore undertook a similar survey of the literature for ethyl methane sulphonate (EMS), the most widely used chemical mutagen. We were limited to studies for which we could determine the number of mutations per survivor, the number of loci at risk, the actual applied dose, and the haploid DNA content of the test organism. Dose estimation for EMS posed problems because we do not know how much EMS reached the DNA in active form: the chemical might have penetrated to the DNA in different ways in different organisms or might have been differently metabolised. Furthermore, the duration of EMS treatment varied as did the methods and conditions of the exposure. For our purposes, therefore, we assumed a uniform distribution of the chemical within the organism at the applied dose. In the case of the mouse, the injected dose of 200 mg kg^{-1} became 200 mg l^{-1} or 1.6×10^{-3} M. To provide a consistent measure of dose, it was necessary to use only concentration in all cases, rather than the product of concentration and the duration of exposure which was often unknown. The data are shown in Table 1.

To obtain the best fit of a curve representing the relationship between mutation rate and genome size, we used the method of least squares on the unweighted data or the unweighted logarithms of the data. In the former case the line of fit deviates markedly from the points for organisms with smaller genomes. Thus the logarithmic values are the only ones reported This represents a fit to the equation $y=mx^b$ where y is the mutation rate and x is the genome size. The

Fig. 1 Rate of X-ray-induced mutation in organisms with different haploid genome sizes. The human point is not experimental.



lines shown in Figs 1 and 2 were obtained in this way. Figure 1 shows the data of Abrahamson *et al.*, for rates of X-ray-induced mutation, except that a few points have been replotted at DNA values we believe to be more accurate. The line has the parameters of $m=1.20\times10^{-14}$ mutations per locus per rad per dalton and $b=0.82$ ($r=0.97$). The EMS results are given in Fig. 2 In this case the line has the parameters $m=2.24\times10^{-14}$ mutations per locus per M per dalton and $b=1.03$ ($r=0.94$). We do not regard either of the values of b as being significantly different from 1.0 given the inhomogeneity of the data.

The relationship between mutation rate and genome size is as striking for EMS as it is for radiation, thus confirming the reality of the ABCW relation. This relation was so unexpected that we could not exclude the possibility that it was coincidence Since the EMS data involve several organisms, loci and investigators not associated with the radiation data (although there are some of each in common), it seems most unlikely that the relationship is a statistical artefact. Accordingly it is proper to consider both the possible mechanisms that may underlie the relation and its significance for estimating human risk from chemical mutagens.

There are at least three possible ways of explaining the ABCW relationship, in which we include now EMS as well as X rays (and, presumably, many or all mutagens). These possibilities, which are not mutually exclusive, are: (1) that the target for mutation is roughly proportional to genome size, (2) that the efficiency of repair of genetic damage is inversely proportional to genome size, and (3) that the size of the mutational event is proportional to genome size. We assume, of course, that the initial lesion (for example, an alkylation) is the same but that the fate of the lesion might differ. It is conceivable, for example, that the average size of interstitial chromosomal deletions could bear some relation to genome size because two different parts of the same chromosome must be in close proximity. Thus if the condensation pattern of chromosomes resulted in proportionately longer folds or gyres in organisms with larger genomes, then the effectiveness of each lesion at producing forward mutations would increase in proportion to genome size and could account for the ABCW relationship.

The second possibility is that the effectiveness of repair is inversely proportional to genome size. This would arise if, for example, the absolute amount of repair were the same in all organisms (even though the total amount of damage is directly proportional to DNA content).

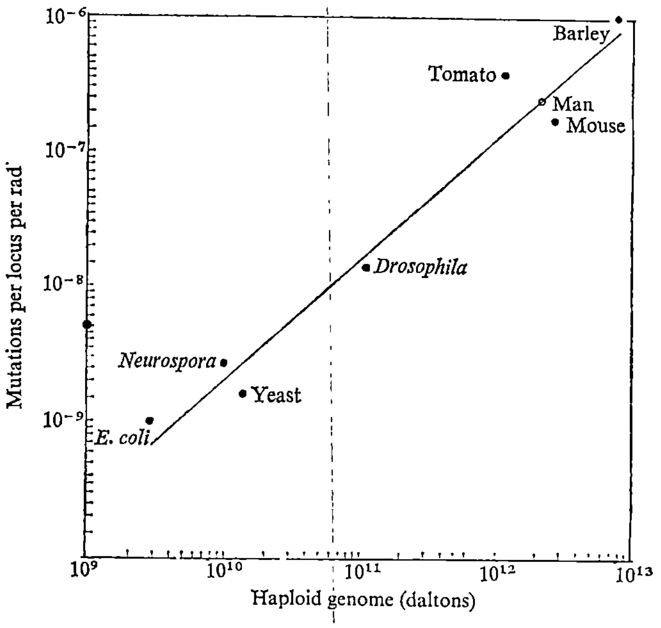


Fig. 2 Rate of ethyl methane sulphonate induced mutation rate in organisms with different haploid genome sizes. The human point is not experimental

The remaining possibility that we have considered is that gene size differs systematically. A similar possibility has been raised, for example, from the correlation between the number and location of salivary gland chromosome bands and gene loci in *Drosophila*[2]. Various models for the organisational and control of genes in higher organisms fit rather well with such a model in those cases where each structural gene is part of a larger genetic unit that includes controlling DNA[2-4]. Others have concluded that among higher organisms, specifically man and chimpanzee, the differences cannot be accounted for primarily by differences in structural genes but that differences in the controlling genes are involved[5]. Such models predict an increase in forward mutation rates with genome size since hits on controlling DNA could lead to loss of function. One test of this

Table 1 Data used to calculate the values in Fig. 2

Organism	DNA per haploid genome (daltons)	Specific locus mutation rate	Genes mutated	Dose of EMS	Duration of treatment	Specific locus mutations per mol of EMS	References
E. coli (bacterium)	2.8×10^{9}*	3.12×10^{-5}†	*tsx* (resistance to phage T_6)	0.1 M	8–15 min	3.1×10^{-4}	*8, †9
		3.8×10^{-5}†		0.2 M	10 min	1.9×10^{-4}	
Neurospora crassa (fungus)	1×10^{10}*	7.25×10^{-6}†	*ad-3A* and *ad-3B*	9.5×10^{-2} M	60 min	7.6×10^{-5}	*10, †11
Schizosaccharomyces pombe (yeast)	1.4×10^{10}*	3.9×10^{-4}†	ad_6 and ad_7	1 M	2 h	3.9×10^{-4}	*12, †13
Drosophila melanogaster (fruit fly)	1.1×10^{11}*	5.7×10^{-5}†	10^3 loci for sex-linked recessive lethals‡	2.5×10^{-2} M	Mutagen injected	2.3×10^{-3}	*1, †14, ‡3, 15
Arabidopsis thaliana	1.6×10^{11}*	7.8×10^{-5}†	Four loci for thiamine auxotrophy	4.1×10^{-3} M	15 h	2.0×10^{-2}	*16, †17
Phaseolus vulgaris (bean)	1×10^{12}*	1.10^{-2}†	Gene for white seed-coat colour	0.04 M	6 h	2.5×10^{-1}	*16, 18
Homo sapiens (man)	2.16×10^{12}*						*19
Cricetulus griseus (Chinese hamster cell cultures)	2.51×10^{12}*	4.46×10^{-4}†	azg^5	1×10^{-2} M	2 h	4.4×10^{-2}	*19, †20
Mus musculus (mouse)	2.57×10^{12}*	7.1×10^{-5}§	Eight specific loci	200 mg kg^{-1} or 1.6×10^{-3} M	Mutagen injected	4.4×10^{-2}	*19
Zea mays (corn)	5×10^{12}*	1.53×10^{-2}†	*C*, *sh* and *wx*	0.028 M	5 h–5 d	5.5×10^{-1}	*16, †21

Within each line asterisks, daggers and double daggers identify the appropriate references.
§ Personal communication from R. B. Cumming.

Table 2 Forward mutation rates induced in prokaryotic organisms

Organism	Genome size (daltons)	Radiation Observed values	Radiation Extrapolated values	EMS Observed values	EMS Extrapolated values	References
E. coli	2.8×10^{9}*	1×10^{-9}†	6.74×10^{-10}	2.5×10^{-4}	6.75×10^{-5}	*8, †9
T_2	1.2×10^{8}*	7.8×10^{-13}†	3.2×10^{-11}	7×10^{-3}‡	1.8×10^{-6}	*22, †23, ‡24
T_4	1.5×10^{8}*	1.08×10^{-8}†	4.5×10^{-11}	–	3×10^{-6}	*22, †25
φX174 and S13	1.7×10^{8}*	–	7.91×10^{-13}	2.91×10^{-16}†	2.38×10^{-8}	*26, †27

Asterisks, daggers and double daggers apply as in Table 1.

explanation would be to measure mutation rates in conditions such that only changes in the structural gene are detected in organisms of different genome size. Another prediction from these models is that the mutation rate will be determined mostly by the size of the structural gene when the proportion of controlling DNA is small. Thus one would expect that organisms with most of their genomes consisting of structural genes would have similar mutation rates, presumably much like that of *E. coli*. Although we have not been able to find much data on this matter, Table 2 shows that neither this prediction nor an extrapolation of the ABCW relation seems satisfactory.

The correlations, regardless of the nature of the mechanism underlying them, provide support for the proposal to express the exposure to chemical mutagens as an equivalent radiation dose. One of the major difficulties in the regulation of human exposure to mutagens is the quantitative estimation of the genetic risk. The most thorough assessment of mutagenicity has been carried out for ionising radiation and maximum population exposure limits have been laid down. On this basis a unit which could be used to quantify human exposure has been suggested, the rem-equivalent-chemical (rec or "radequiv.") which is defined as that concentration of a chemical mutagen that produces an amount of genetic damage equal to that produced by 1 rem of chronic irradiation[6,7]. For such a unit to be meaningful the ratio of mutagenicity of the chemical to radiation must be the same for man and the test organisms. The curves in Figs 1 and 2 indicate that there is a remarkably constant relationship over several orders of magnitude of mutation rate, although there is some uncertainty in the data for any one organism. Since the lines of best fit have somewhat different slopes (1.1 and 0.9) we have used the ratio of the fitted values at the geometric mean (*Arabidopsis*) to calculate the rec value of 4×10^{-6} M. Expressed in terms of rads, therefore, treatment by a 1-M solution is equivalent to about 2.5×10^{5} rad.

There are, of course, other problems in estimating the human risk and there is the problem of assessing the benefit of the chemical also. Nevertheless, if similar correlations are found for other mutagens, rough extrapolations from the simple, rapid microbiological screens to human risk can be made to provide a quantitative evaluation. This would be useful in planning mammalian experiments and evaluating the results. Mammalian systems will still be important because of the potential influence of uniquely mammalian aspects of metabolism, absorption, and excretion of compounds and the possibility of classes of damage that do not occur in prokaryotes, such as chromosome breakage and non-disjunction. Nevertheless, the rec concept has many possible uses in the light of the data presented here which suggest that there is a relatively constant value of rec for all organisms.

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Stimulation of mouse lymphocytes by insoluble anti-mouse immunoglobulin

It is generally agreed that B-cell surface immunoglobulin (Ig) functions as the receptor for antigen, and so accounts for the specificity of the immune response by clonal selection. It is not known, however, how the combination of surface Ig with antigen stimulates the cell to proliferation and/or differentiation to secrete antibody at a high rate[1]. A signal could be delivered directly through the receptors, either by an allosteric transition in the receptor itself or by cross-linkage or aggregation of receptor Ig in the fluid cell membrane, analogous to antigen-induced or anti-IgE-induced histamine release from mast cells[2]. Alternatively, the receptor may act passively as an antigen-specific 'address' for the delivery of an antigen-associated non-specific (that is, potentially polyclonal) signal to some other site on the cell surface[3].

To test whether optimal cross linkage of receptor Ig can stimulate B cells, I have coupled purified rabbit anti-mouse Ig (predominantly anti-κ specificity) covalently to the surface of polyacrylamide beads. The anti-Ig beads were added to normal mouse spleen cell cultures. As Fig. 1 (solid bars) and Table 1 show, anti-Ig beads are strong polyclonal mitogens for normal mouse spleen cells; they stimulate a 10–20-fold increase in incorporation of ^{125}I-iododeoxyuridine (IdUrd) at 48 and 72 h. Control beads prepared with purified rabbit anti-*p*-azobenzenearsonate (anti-ars) antibodies had no effect (Fig. 1 and Table 1). Isotope incorporation data were confirmed by direct microscopic inspection: the number and percentage of blast cells in anti-Ig bead-stimulated cultures were ten times greater than in controls. The blasts were B cells as shown by positive immunofluorescent staining for surface Ig, while parallel phytohaemagglutinin-stimulated cultures yielded surface Ig negative blasts.

This is the first report of significant stimulation of mouse B lymphocytes by purified anti-mouse Ig. Since soluble anti-Ig stimulates rabbit[8], pig[9] and chicken[10] lymphocytes, the failure of soluble anti-Ig to stimulate lymphocytes of other species remains a puzzle[1]. Insolubilisation on a surface has been shown to convert two other mouse B-cell ligands, which in soluble form are mitogens for T cells only—concanavalin A[11] and phytohaemagglutinin[12]—into B-cell mitogens, and to convert haptens or thymus dependent antigens into thymus independent antigens[13]. Insolubilisation on a surface creates a matrix of ligands at a fixed density, prevents internalisation and affects redistribution on the cell surface (although it does not prevent capping of receptors and release from the surface[14]). Which of these effects is crucial in this system remains to be determined.

A high density of anti-Ig on the bead surface was required for stimulation (Table 1). The peak response occurred with 64 and 130 μg of antibody per ml of beads. A concentration of 400 μg ml^{-1} stimulated suboptimally, whereas 6 μg ml^{-1} and 1 μg ml^{-1} failed to stimulate. Five times fewer beads per culture gave half the response, without a change in the optimal density per bead (data not shown). Since the beads (BioGel P30) exclude Ig from the interior, all the anti-Ig was at or near the bead surface. Beads are nearly close packed at 400 μgm l^{-1} if one assumes the limit of a smooth spherical surface of radius 50 μm. This rather sharp dependence on ligand density is expected if the receptor Ig signal to the interior of the cell depends on the formation of small local aggregates of receptors, as seems to be the case with mast cell triggering[15]. To induce receptor aggregates on the cell surface, the anti-Ig molecules must be close together on the semi-rigid bead surface, since the density of adjacent pairs (or trios) of anti-Ig molecules would vary nearly as the square (or cube) of the anti-Ig density, if the distribution of anti-Ig on the bead surface is random.

Taken together with the extensive studies of the effect of density of antigenic determinants and molecular structure on the immunogenicity of thymus-independent antigens[16], these results support the view that one route to B-cell activation is a critical extent and duration of cross linkage of cell surface

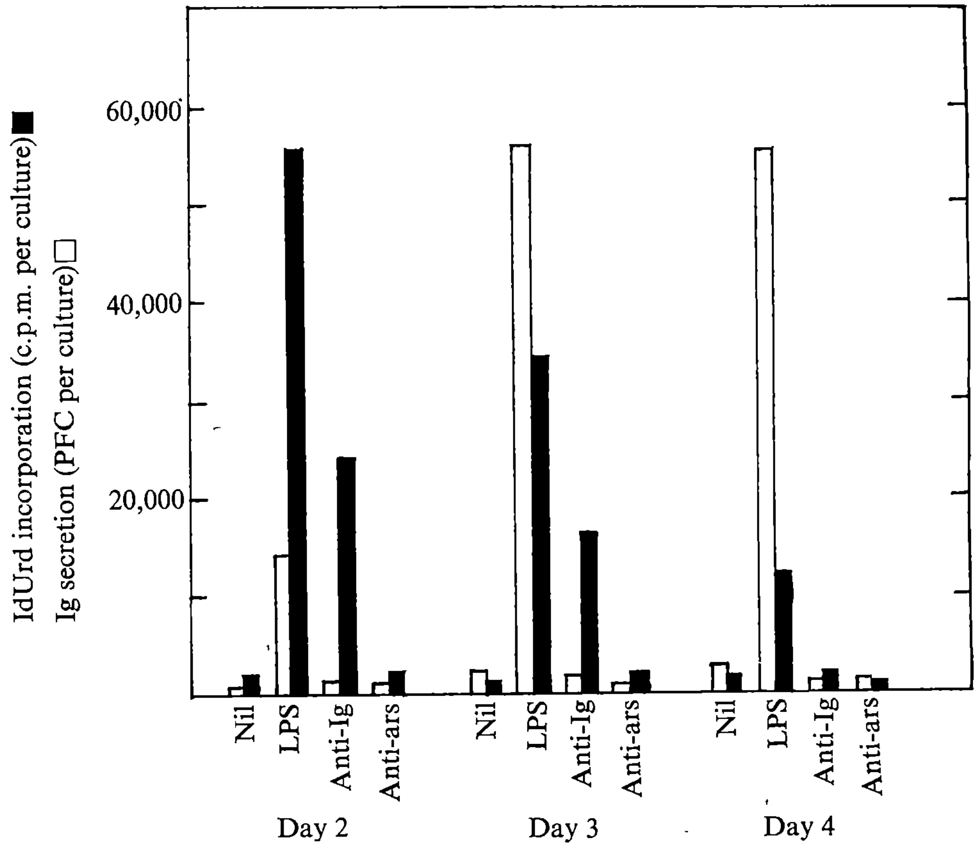

Fig. 1 Comparison of proliferation (solid bars, ^{125}IdUrd incorporated) and number of Ig-secreting cells (open bars, PFC) in normal spleen cell cultures stimulated with lipopolysaccharide (LPS, from *E. coli* 055:B5, Difco, 100 μg ml^{-1}) or antibody-coupled polyacrylamide beads (130 μg ml^{-1} settled beads; 5 × 10^3 beads per culture). Values shown are the arithmetic means of triplicate cultures. Standard errors were of the same order as those shown in Table 1. Normal 8–12-week CBA mouse spleen cells were washed with medium and cultured at 5 × 10^5 cells per 200 μl of modified Eagle's medium containing extra glutamine, antibiotics and 10% heat inactivated foetal calf serum in flat bottom wells in micoplates (Linbro IS-FB-96) in an atmosphere of 10% CO$_2$ in air. 1 μCi ^{125}IdUrd (100 μCi μg^{-1}, Radiochemical Centre, Amersham), was added to some cultures 4 h before collection on to glass fibre filters. Parallel cultures were collected with a Pasteur pipette, washed twice, and assayed for PFC using the plaque assay described below The anti-mouse Ig antibodies were isolated from pooled hyperimmune antisera to normal mouse Ig and to a γ2a,κ and a γ1,κ myeloma protein, using a Sepharose immunoadsorbant, and eluted with glycine-NaCl buffer, *p*H 2.4. The immunoadsorbant (loaned by D. Kilburn) was coupled with a mouse κ chain dimer isolated from the ascites fluid of mice bearing MOPC 70A plasmacytoma. Hyperimmune anti-ars antisera were raised in rabbits to a haemocyanin azo conjugate, and the antibodies were purified on arsanilate Sepharose. The purified anti-Ig antibodies were trace labelled with ^{125}I by a modification of the Chloramine T technique[4]. Antibody was coupled to polyacrylamide beads (BioGel P30, 100–200 mesh, BioRad) by the acyl azide procedure of Inman and Dintzis[5]. The hydrazide intermediate (0.75 mmolg^{-1}) was reacted with 0.15 M NaNO$_2$ in 0.3 M HCl for 3 min. After washing as described, the beads were added to an equal volume of antibody in 0.09 M sodium tetraborate, *p*H 9, and tumbled slowly overnight at 4 °C. Unreacted azide groups were reconverted to the amide. The beads were washed extensively with 6% saline *p*H 8.6, followed by sterile phosphate-buffered saline, *p*H 7.2, in which they were stored at 4 °C with azide. Ten per cent of the antibody added at 1 mg ml^{-1} beads was coupled to the beads by this procedure. Before use they were washed extensively with sterile saline and then culture medium. Immunoglobulin-secreting cells were counted using an adaptation of the reverse haemolytic plaque assay developed by Molinaro[6]. Sheep red cells were coated with anti-mouse Ig (anti-κ) using hybrid antibody[6], and used in a monolayer plaque technique[7]. Normal spleen contained 4,000–10,000 direct PFC per 10^6 nucleated cells. In LPS-stimulated cultures, about 20% of the cells were PFC at days 3 and 4. In general, anti-Ig coated red cells gave 1,000 times the number of direct (anti-sheep red cell) plaques obtained with uncoated red cells.

Table 1 Effect of surface density of anti-Ig on stimulation

	^{125}IdUrd incorporated (c.p.m. per culture±s.e.)	Ig-secreting cells (PFC per culture±s.e.)
Nil	1,190±50	3,480±260
LPS (100 μg ml^{-1})	17,300±540	63,300±2,400
Unmodified beads	1,200±80	1,690±640
Anti-Ig beads 1 μg ml^{-1}*	1,070±30	3,480±140
6 μg ml^{-1}	1,254±30	1,720±290
64 μg ml^{-1}	22,180±1,310	5,690±350
64 μg ml+LPS	17,970±2,110	63,900±13,140
Nil	1,350±30	2,270±180
LPS (100 μg ml^{-1})	34,870±1,350	56,130±1,540
Anti-Ig beads 64 μg ml^{-1}	15,040±1,250	1,030±30
130 μg ml^{-1}	16,730±2,390	1,450±130
400 μg ml^{-1}	5,450±770	1,120±280
Anti-ars beads 400 μg ml^{-1}	634±15	1,660±280

Culture conditions were the same as Fig. 1. Cultures were collected on day 3. Arithmetic means±the standard error of the mean for triplicate cultures are shown.

*μg of purified antibody per ml of settled beads (10^6 beads per ml). 5×10^3 beads, enough to form a layer two beads thick on the bottom of the well, were added to each culture.

structures, one of which is receptor Ig. But two alternative explanations must be considered. The first is that the anti-Ig beads stimulate B cells indirectly, by stimulating a surface Ig-bearing, non-B cell (macrophage or activated T cell) to release a B-cell activating factor. The second is that the Fc region of the surface-bound anti-Ig delivers an activating signal to the B cell through its Fc receptors. This is unlikely since antigen–antibody complexes[17] and beads densely coupled with rabbit anti-hapten antibody (Table 1) do not stimulate mouse spleen cells. These alternatives are being tested using purified B cells and beads conjugated with the (Fab$'$)$_2$ fragment[18] of anti-Ig. The polyacrylamide bead itself is unlikely to interact with any cell surface structure or deliver any signals to the cell. First, it is the substrate of choice for cellular immunoadsorbants since, unlike Sephadex, glass beads or other plastics, it does not adsorb sticky cells nonspecifically, as shown by 99±1% yields of passed cells in column fractionation procedures[19]. Second, it is not a polyclonal activator as shown by normal requirements for antigen and accessory cells in the antibody response to sheep erythrocytes of mouse spleen cells cultured directly on a polyacrylamide surface in a microwell technique[20]. Third, unmodified polyacrylamide beads or beads coated with rabbit anti-hapten antibodies failed to stimulate cells in the experiments reported here and elsewhere[13]. Sephadex G-15 beads coupled[21] with anti-Ig at 30 μg ml^{-1} and 170 μg ml^{-1} failed to stimulate, although quantitation was difficult because the beads bound considerable ^{125}IdUrd in the absence of cells. The lack of stimulation may have been due to the wrong surface density of anti-Ig or to interaction of the Sephadex with the cell surface.

Although the anti-Ig beads were comparable with LPS in their ability to stimulate cell proliferation, it was striking that unlike LPS or other B-cell mitogens, with the exception of dextran sulphate[22], they failed to stimulate significant increases in numbers of immunoglobulin-secreting cells (Fig. 1 and Table 1). Since the reverse haemolytic plaque assay (see Fig. 1 legend) might be relatively inefficient for IgG and IgA-secreting cells, the blasts in bead-stimulated cultures were examined for intracellular Ig using a polyvalent fluorescent anti-Ig antiserum, and found to be negative. This result has several possible interpretations: (1) The anti-Ig beads may be turning off Ig synthesis in the stimulated cells. I have found that free anti-Ig inhibits plaque-forming cells without much effect on IdUrd incorporation in LPS-stimulated cultures (data not shown) in agreement with Andersson *et al.*[23] who pretreated cells with anti-Ig at 4 °C before culturing with LPS. The anti-Ig beads, however, did not turn off Ig secretion in cultures stimulated with LPS (Table 1). (2) T cells or macrophages may be inhibiting Ig secretion. (3) The anti-Ig beads may be giving a proliferation signal to a subpopulation[24] of B cells which requires a second differentiation signal to proceed to a high rate of Ig synthesis and secretion[25,26]. Soluble anti-Ig stimulates proliferation of rabbit lymphocytes by cross linking receptors, but antibody secretion depends on the addition of T-cell factors[27]. This radical dissociation of proliferation from immunoglobulin secretion in anti-Ig bead-stimulated B cells will prove useful as a model for regulation of antibody synthesis in antigen-activated B cells in the mouse, where the dissection of interacting components in the immune response has proceeded much further than in other species.

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Identical sequence of light chains from rabbit anti-streptococcal antibodies

CONSIDERABLE advances have been made with special breeding programmes in the selection of rabbits with amplified immune responses to polysaccharides specific for different streptococcal groups[1,2]. Levels of group specific antibodies of 50 mg ml^{-1}

κ chain NH_2–terminal residue positions

	0	1	2							10										20	
K9-106	.	.	Ile	Val	Met	Thr	Gln	Thr	Pro	Ser	Ser	Lys	Ser	Val	Pro	Val	Gly	Asp	Thr	Val	Thr	Ile
K9-335	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K9-338	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K29-213	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K27-306	.	.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K6-141	.	.	—	—	—	—	—	—	—	—	●	—	—	—	—	—	—	—	—	—	—
Rabbit b4	Ala	S	V	S	—	—	—	—	—	S	S	S	S	S	S	—	—	S	—	—	—

								HV$_I$													
				25					30					35					40		
K9-106	Asn	Cys	Gln	Ala	Ser	Gln	Ser	Val	Tyr	Ser	Asn	Asn	Arg	Leu	Ala	Trp	Phe	Gln	Gln	Pro	Lys
K9-335	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K9-338	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K29-213	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
K27-306	—	—	—	—	—	—	—	—	—	—	—	—	(—)	—	—	—	—	—	—	—	—
K6-141	—	—	—	—	—	—	—	—	—	—	Asx	Asx	—	—	—	—	—	—	—	—	—
Rabbit b4	—	—	—	—	—	—	—	V	V	V	V	V	V	—	S	—	S	—	—	—	—

Fig.1 The amino-terminal sequences of rabbit allotype b4κ light chains isolated from antibodies to the streptococcal polysaccharide. Solid lines indicate sequence identity to the leading sequence. Asx, undetermined asparagine residue. HV$_I$ marks the first hypervariable region[14]. For comparison a prototype rabbit b4 sequence is indicated[2]. S indicates positions that can express subgroup specific residues; V indicates highly variable positions. Dots mark deletions.

have been reported. Such antisera are valuable because they often contain monoclonal antibody which can be used in studies of the origin of antibody diversity. These antibody probes have stimulated a search for variable region markers common to antibodies specific for the same polysaccharide antigen[3] and produced by closely related rabbits[4,7]. In a continuation of earlier marker analyses[3] and of a breeding programme for restricted high responders[8], we have used the streptococcal group A variant antigen (strain A486 variant of the Rockefeller University) to immunise part-inbred rabbits[6,7]. This cell wall polysaccharide is a homopolymer of rhamnose[9,10] of very limited heterogeneity. We report the partial amino acid sequences of light chains of the b4 allotype isolated from monoclonal antibodies raised in six closely related rabbits after a primary or secondary course of intravenous immunisation with a streptococcal group A variant vaccine[6].

The light chains were selected for their amino-terminal isoleucine residue. Previous work had shown that such light chains belong to a separate subgroup which accounts for no more than 3% in a rabbit immunoglobulin pool[11,12].

Rabbits K9-106, K9-335 and K9-338 were littermates from a mating of unrelated parents (K429-1♂ × K23♀), rabbit K6-141 arose from a brother–sister mating of K19♀ × K20♂, which were littermates to K23; rabbits K29-213 and K27-306 were progeny of K9-106 with its siblings K9-107 and K9-133.

Partial amino acid sequence analyses were performed with a Beckman 890B sequencer. Both partially reduced and alkylated light chains and fully reduced and alkylated light chains were used. Fully reduced samples were alkylated with ^{14}C-labelled iodoacetic acid (Radiochemical Centre, Amersham). The quadrol program was used for all analyses. All phenylthiohydantoin amino acid derivatives were analysed by gas chromatography (Beckman GC45 gas chromatograph)[12], by thin-layer chromatography on silica gels with identification by the starch–iodine method[13], and by amino acid analyses after back-hydrolysis with hydroiodo acid to free amino acids[12].

Figure 1 shows the sequences of the six light chains up to position 42. They include framework residues (positions 2–28 and 36–42) and the entire first hypervariable region (positions 29–35)[14]. The principal conclusion is the identity in sequence from positions 2–42. In contrast, the hypervariable regions from two other light chains isolated from two anti-azobenzene arsonate antibody fractions produced by one rabbit[15] differed at positions 30–34, although they belonged to the same subgroup. Also light chains of separate subgroups that were isolated from antibodies to *p*-azobenzoate[16], pneumococcal type III polysaccharide[17] and streptococcal group C polysaccharide[18] differed significantly in the first hypervariable region. It is therefore likely that the unique sequence of the first hypervariable region of these light chains correlates with the antigen specificity. Light chains of this subgroup were found previously in streptococcal group C specific antibodies[11]. The amino acid sequences of their first hypervariable regions are not yet available.

Valine at position 11 and glycine at position 17 were designated species-specific for rabbit κ chains of the b4 allotype because no alternative amino acids were found in these positions in rabbit light chain sequences available at that time[11]. As such, they served in arguments about the diversification of rabbit light chains as the basis for a proposed gene expansion and contraction mechanism[11,19]. But, the finding of lysine at position 11 and aspartic acid at position 17 in six rabbit light chains from allotype b4 rabbits shows that these positions are not occupied by species-specific amino acid residues that could serve as markers. Rather, it is likely that they mark subgroup-specific positions characteristic for the amino terminal sequence Ile–Val–Met. None of the light chains was associated with a particular heavy chain allotype; they occured either with heavy chains of the group *a2* marker (K9-106, K9-335, K9-338 and K29-213) or with the group *a3* marker (K27-306 and K6-141). Accordingly they were found in antibodies of variable and overlapping isoelectric points.

All but light chain K6-141 were associated with dominant clonotypes. The dominant clonotype of rabbit K6-141 anti-

A-variant polysaccharide antibodies at the peak of the antibody response was characterised by an amino-terminal sequence of Ala–Leu–Val. This sequence is characteristic of another subgroup of rabbit antibody light chains[11,12].

Barstad *et al.* showed identical amino-terminal sequences of three light and five heavy immunoglobulin chains from myeloma proteins of BALB/c mice with binding activity to phosphorylcholine[20]. Our finding of sequence identity from residues 1–41 in light chains produced by closely related but not inbred rabbits, in response to streptococcal A variant polysaccharide suggests that these variable light chain regions are the products of a germ line gene, and not generated by random somatic hypermutation and subsequent selection. A fundamental insight into the selection of antibody light chains to streptococcal polysaccharide antigens which share a rhamnose backbone[1,9] can be expected from the sequence analysis of light chains derived from anti-streptococcal group C antibodies with the amino-terminal sequence Ile–Val–Met[11].

Our data also suggest that single rabbits (for example, K6-141) can respond to the streptococcal group A variant polysaccharide with antibodies containing light chains of two separate subgroups. This is not consistent with the view that a basic nucleotide sequence serves as a germ-line gene-encoded matrix from which new structural genes are derived by subsequent step mutations. It rather suggests two separate germ line genes for one antigen.

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Syntheses and immunological evaluation of bovine proinsulin C-peptide analogues

PROINSULIN is a single-chain polypeptide in which the A and B chains of insulin are linked together by the connecting peptide[1]. The terminals of the connecting peptides are occupied by the basic dipeptides, Arg–Arg and Lys–Arg, respectively, and proinsulin C-peptide is defined as connecting peptide minus the basic dipeptides. The immunological reactivity of synthetic [59-formyllysine]-bovine proinsulin 31–60, which comprises the entire sequence of the connecting peptide segment of bovine proinsulin, is indistinguishable from that of natural bovine proinsulin when compared on an equimolar basis[2]. The immunoassay system involved a bovine proinsulin antiserum[3], which was purified by the removal of the antibodies directed towards the insulin region of the molecule. We have now evaluated the structural features of bovine connecting peptide segment which determine its reactivity with bovine proinsulin antisera. For this purpose, homogeneous synthetic peptides of defined structures serve as excellent substrates. We synthesised twelve peptides related to bovine proinsulin C-peptide which has the following amino acid sequence

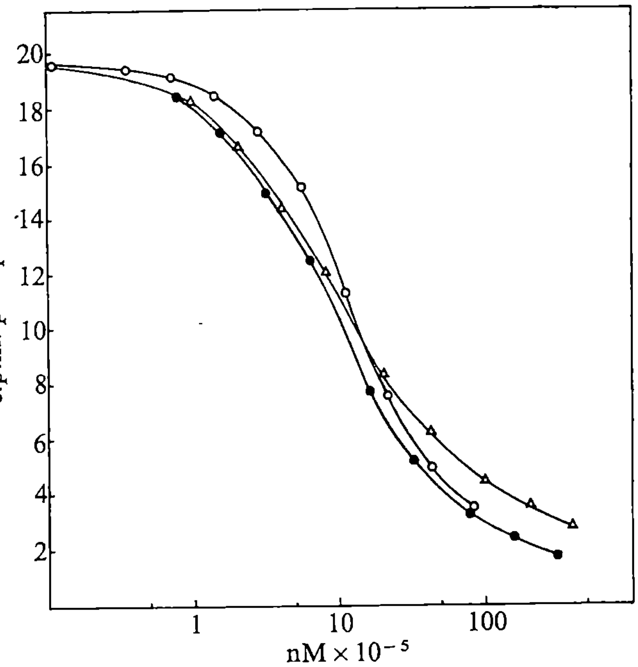


Fig. 1 Double-antibody radioimmunoassay using bovine proinsulin antiserum and ^{125}I-tyrosylated [59-formyllysine]-bovine proinsulin 31–60 as tracer. ●, Synthetic [59-formyllysine]-bovine proinsulin 31–60; △, natural bovine C-peptide; ○, natural bovine proinsulin.

33
Glu–Val–Glu–Gly–Pro–Gln–Val–Gly–Ala–Leu–Glu–Leu–Ala–

58
Gly–Gly–Pro–Gly–Ala–Gly–Gly–Leu–Glu–Gly–Pro–Pro–Gln

The peptides were constructed entirely by the fragment condensation method in solution using catalytic hydrogenolysis to remove the benzyloxycarbonyl group as amino protection. Mixed anhydrides[4] or azides[5] of protected di-, tri- and tetrapeptides were used for acylation. The final products were purified exclusively by CM-Sephadex (free form) column chromatography using increasing concentrations of acetic acid for elution. Each of the ensuing products was homogeneous as judged by thin-layer chromatography, and acid hydrolysates of these peptides contained the constituent amino acids in the ratios predicted by theory. The synthetic bovine C-peptide fragments were as follows: bovine proinsulin (BP) 33–46, BP 33–50 BP 33–53, BP 33–54, BP 37–46, BP 37–49, BP 37–50, BP 37–52, BP 37–53, BP 39–52, BP 41–53 and BP 41–50.

Immunological reactivities of these synthetic peptides were determined by the double antibody method[3] using an antiserum against natural bovine proinsulin raised in a rabbit and ^{125}I-synthetic tyrosylated [59-formyllysine]-bovine proinsulin 31–60 as tracer. The tyrosylated derivative was obtained by the interaction of benzyloxycarbonyltyrosine N-hydroxysuccinimido ester[6] with [59-formyllysine]-bovine proinsulin 31–60 followed by hydrogenolysis. This peptide was iodinated by a modification of the method of Frechet *et al.*[7].

Figure 1 shows the relative immunoreactivity of natural bovine proinsulin and C-peptide and synthetic [59-formyllysine]-bovine proinsulin 31–60. The result indicates that the synthetic peptide reacts similarly to endogenous bovine proinsulin and C-peptide on an equimolar basis.

The displacement curves of the synthetic bovine proinsulin C-peptide fragments are shown in Fig. 2. All the peptides

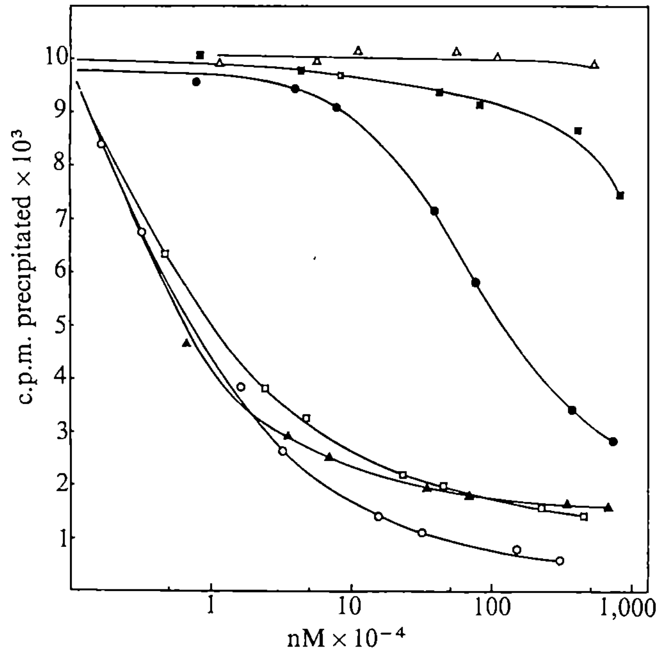
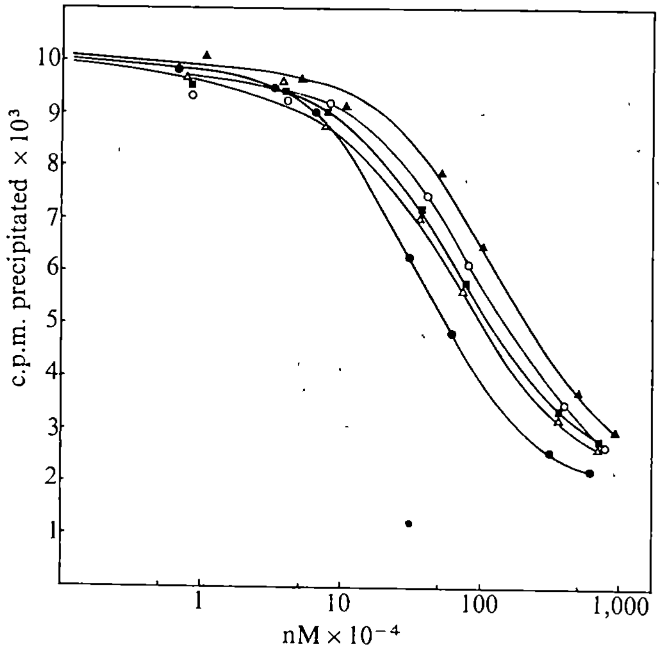


Fig. 2 Double-antibody radioimmunoassay of synthetic peptides related to bovine proinsulin C-peptide using bovine proinsulin antiserum. ^{125}I-tyrosylated [59-formyllysine]-bovine proinsulin 31–60 was used as tracer. ○, [59-Formyllysine]-bovine proinsulin 31–60; ▲, BP 33–46; □, BP 33–50, BP 33–53, and BP 33–54; ●, BP 37–46, BP 37–49, BP 37–50, BP 37–52, and BP 37–53; ■, BP 39–52; △, BP 41–50 and BP 41–53.

that contained the amino acid sequence corresponding to the bovine proinsulin 33–46 fragment were found to cross react almost identically with synthetic [59-formyllysine]-bovine proinsulin 31–60. Displacement curves of BP 33–50, BP 33–53 and BP 33–54 were plotted as one representative line because of their similar reactivities. BP 37–46, BP 37–49, BP 37–50, BP 37–52 and BP 37–53 displaced the tracer similarly, as shown by the mean of their curves. These peptides, however, exhibited significantly reduced cross reactivity. The shorter peptides, BP 39–53, still reacted though very weakly, whereas neither BP 41–50 nor BP 41–53 competed with the tracer at the concentrations we used. The marked difference of the cross reactivities of BP 33–53, BP 37–52, BP 39–52 and BP 41–53 clearly indicates the effect of decreasing the chain length in that part of the molecule which contributes to the interaction with its antibody. Our investigation shows that the major antigenic region in the connecting peptide portion of bovine proinsulin is located within residues 33–46 in the amino acid sequence. The evidence was obtained with the major anti-C-peptide antibody but certainly not the only one in the antiserum studied. Removal of four amino acid residues, $\overset{33}{\text{Glu}}$–Val–Glu–$\overset{36}{\text{Gly}}$, resulted in a marked decrease in immunoreactivity. The proline residue at position 37 seems to be of special importance. Peptides, BP 39–52, BP 41–50 and BP 41–53 which do not contain the proline residue at position 37 show extremely low reactivity or do not react at all. Figure 3 represents individual displacement curves of BP 37–46, BP 37–49, BP 37–50, BP 37–52 and BP 37–53. There is a gradation of increasing reactivity with increase of the chain length from the carboxyl end of BP 37–46. A partial contribution of the 47–60 sequence on the immunological reactivity has been suggested from our observation[2] that an inversion of residues 50 and 52 in the bovine proinsulin connecting peptide segment resulted in a distinct loss of immunoreactivity. The observations reported here also indicate that the 46–53 sequence participates in binding, although this contribution is rather small.

Fig. 3 Competition between BP 37–46 (▲), BP 37–49 (○), BP 37–50 (■), BP 37–52 (△), and BP 37–53 (●) and ^{125}I-tyrosylated [59-formyllysine]-bovine proinsulin 31–60 for binding to bovine proinsulin antiserum.



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Errata

In the article "Scotopic and photopic dark adaptation of the b wave in isolated rat retina" by W. Ernst and C. M. Kemp (*Nature*, **258**, 170; 1975) the sentence that begins in line 17 of the second paragraph should read ... This means that either bleaching has caused some permanent change in the way light of different wavelengths reaches the rhodopsin molecules, or the b-wave cells are controlled by a non-rhodopsin system. ... and not as printed.

The correct authorship of the article "Synthesis mimics of insect juvenile hormone" (*Nature*, **232**, 486; 1971) should be

Ferenc M. Pallos
Hwalin Lee
Peter E. Letchworth
John B Miaullis
Julius J. Menn

and not as printed in the original article or in the corrigendum which appeared in *Nature* (**253**, 288; 1975).

Hazards of life

Natural Hazards: Local, National, Global. Edited by Gilbert F. White. Pp. xvi+288. (Oxford University: New York, London and Toronto, 1975.) £7.50. *Geological Hazards: Earthquakes - Tsunamis - Volcanoes - Avalanches - Landslides - Floods.* By B. A. Bolt, W. L. Horn, G. A. Macdonald and R F. Scott. Pp. viii+328. (Springer: Berlin and New York, 1975.) *Physical Aspects of Natural Catastrophes.* By Adrian E. Scheidegger. Pp. xiii+289. (Elsevier: Amsterdam, Oxford and New York, 1975). Dfl. 70; $29.25.

IN 1967 a group of geographers in the US and Canada initiated a study of the human and social responses to natural hazards in different parts of the world. The sort of questions they hoped to answer were: How many people inhabit particular areas of hazard and why do they continue to do so? How do these inhabitants perceive the extreme natural events to which they are subject? What options are open to them, both individually and collectively, in adjusting to the dangers and how are these options exercised? For six years, investigators in more than 23 countries collaborated in interviewing samples of populations at risk from earthquakes in San Francisco, cyclones in Bangladesh, drought in Kenya and Australia, floods in India and Britain, volcanic eruptions in Hawaii, avalanches in Norway, and various other hazards in nations at different stages of development.

The results of this 'loosely co-ordinated' survey, many of which are described in the 32 articles in *Natural Hazards*, are disturbing, although perhaps not entirely surprising. Galveston, Texas, is prone to hurricanes; but although most residents of the city are aware of the threat, about a third of them refuse to believe they will ever be affected personally and therefore take no precautions. About a fifth of San Franciscans think they will never experience earthquake damage. Over half of those who live on the River Severn floodplain in Shrewsbury, England, not only do not expect to witness a flood in the future but do not even know they have a flood problem. The list goes on and on; and it all makes fascinating reading.

The point of this study, however, is not to catalogue human ignorance and irrationality for their own sake but to assess the nature of the real responses with which public policy to mitigate the effects of natural hazards must contend. All too often in the past, technological solutions to the problem of coping with hazards have failed because they were based on cost-benefit analyses which assumed that people will always behave in an economically rational way. The cost of flood damage in the US, for example, has continued to increase alarmingly in spite of a multi-billion dollar investment in flood control works spread over more than 20 years. Likewise, hurricane warning systems, however accurate, have been less than completely effective in convincing the public to take avoidance measures. The fact is that real people, given freedom of choice, seldom behave in the tidy way that policy makers would wish. This may be partly through ignorance and partly through misguided optimism, but it also reflects the existence of more than one scale of human values.

The importance of *Natural Hazards* is that it gives substance in a particular context to the old allegation that there is nothing so unpredictable as people and points up the folly of those who have persistently ignored this simple fact of life. The book should therefore be essential reading for all those scientists who see complete solutions to the problems of natural hazards in the rational pursuit of scientific knowledge and to all those politicians and administrators who have been misled into believing that all physical and human nature may ultimately be subdued by the technological fix.

But although the scientific approach may not be sufficient to cope with natural hazards, it is certainly necessary, and is given due prominence in *Geological Hazards.* Presumably the original work of Gilbert F. White and his colleagues is still too new to have reached the secondary texts, and so there is little here about the vagaries of human behaviour. On the other hand, although primarily a book about science, the science is placed firmly in the social context which gives it point. As a general (descriptive and non-mathematical) introduction to a wide range of natural hazards, this book may be recommended without reserve.

With neither the common theme of human response nor the self-justifying interest of the general reader, the linking of different hazards in *Physical Aspects of Natural Catastrophes* has less point. Intended largely as a scientific source book for engineers, the book is mathematical and utilitarian. It may be referred to, if necessary, but is unlikely to be read.

Peter J. Smith

Quantum mechanics

Quantum Mechanics for Organic Chemists. By Howard E. Zimmerman. Pp. x+215. (Academic: New York and London, May 1975.) $16.50; £7.90.

THE first chapter is devoted to the methods and results of the LCAO-MO Huckel approach. Though the first section of this chapter is headed "Orbitals", I feel that a reader of this book would need to know something about atomic and molecular quantum mechanics before he started. It seemed to me surprising to devote so much of the first chapter of a book like this to π-orbitals, though I think the author is very sensible to use the allyl system as his example.

In the second chapter Zimmerman deals with eigenvalues, eigenfunctions operators and the variation method, which is dealt with clearly at some length. The next chapter explains the effects of molecular symmetry and introduces the reader to group theory. After that the book returns to the Hückel method and extends the earlier consideration, continuing with an account of more advanced treatments. Systems are treated as containing many interacting electrons; Slater determinants and the SCF method are described. There is also a description of configuration interaction and examples of its applications.

It is fair to say that this book approaches and tackles the problem of presenting quantum mechanics to organic chemists in a manner different from that used by other books. As such, it is interesting and stimulating; I certainly found it very much worth studying. But I must say also that I was not left convinced that the treatment and sequence used is the best way to tackle the job; but it deserves consideration and parts are well done and most useful.

J. W. Linnett

Giant algal cells

The Physiology of Giant Algal Cells. By A. B. Hope and N. A. Walker. Pp. xiii+201. (Cambridge University Press: London, 1975.) £8.50; $24.95.

THIS small book, on what may at first seem a restricted subject, blossoms to cover a range of electrophysiological phenomena in both animals and plants. The plants concerned (mainly Charophyta, with mention of appropriate Bryopsidophyceae, Chlorococcales and Ceramiales), and also the basic electrophysiological techniques involved, are adequately introduced in the first chapter. The glossy photographs of *Chara, Nitella* and *Griffithsia* could, however, have been replaced by more electronmicrographs.

Chapter 2 provides a chronology of work from 1774 up to 1938, but the subsequent period of consolidation of earlier ideas is what this book is really about. Chapter 3 starts off gently with "Water Relations", although the biophysical leanings of the book are already apparent in the extensive mathematical treatments which place perhaps too much dependence on hieroglyphics (51 special symbols and 32 abbreviations are listed at the front) and which make too little attempt to put important concepts clearly in words, a characteristic of the rest of the book.

In the following chapters little consideration is given to at least one part of the professed readership: undergraduate students; and recommendation of this book to the majority of plant physiology students would lead to nothing short of baffling disaster. The specialist, on the other hand, may be repeatedly frustrated at being sent off to the literature because of the lack of detail in the rather brief text. It is difficult to determine whether the book is meant to be a thorough review article on a very wide-based topic or a half-hearted textbook of plant electrochemistry; the authors have perhaps not succeeded in striking an adequate balance between the two approaches and have obviously been hampered by lack of space. Some topics receive unfortunately little coverage : photosynthesis, for instance, is only mentioned as an adjunct to several other processes which are affected by light, such as cation uptake, and carbon fixation rates are given minimum space.

The final chapter is refreshingly different, providing a delightfully empassioned account of the beauty and mechanism of protoplasmic streaming, the earliest recorded phenomenon in these giant cells and, apparently, the least quantified. Unfortunately, it does nothing to draw to a conclusion the mass of data in previous pages. The lack of such a concluding section is especially serious in a book so full of extremely critical comment on everyones' efforts, including the authors' own researches. **Edward A. Drew**

Special relativity

Special Relativity. (Oxford Physics Series.) By J. G. Taylor. Pp. 106. (Clarendon: Oxford, Oxford University: London, June 1975.) £1.95.

FOR the past three years Professor Taylor has been giving a series of lectures on introductory special relativity to first-year undergraduates at King's College London. The content and spirit of these lectures now appears in a compact and very readable book from the Oxford Physics Series. The approach is a modern mathematical one, but physics students will find little difficulty in following the text. The first two chapters deal with the physical basis of special relativity, such as space and time measurements. The author deliberately adopts the language and concepts of relativity theory at the outset, rather than the traditional method of building them on an erroneous foundation of Newtonian physics. In this way the theory emerges in a very natural and elegant fashion. Starting solely from the constancy of the speed of light, the Lorentz transformation is introduced. The formal treatment which follows is based on the structure of the Lorentz group, and deals skilfully and concisely with all the major areas of interest in relativistic kinematics and dynamics. Students with a knowledge of vector field theory and electromagnetism will benefit most from this particular approach, although this book will prove a useful teaching text in almost any mathematics or physics department.

A final chapter is included on extensions to special relativity Tachyons (hypothetical particles which travel faster than light) are discussed in unusual depth for a course book, and the question of their existence left open. The reader is then taken to the beginnings of the general theory of relativity with a qualitative account of acceleration effects and the equivalence principle. Some problems and their solutions are given at the end.

In summary, Professor Taylor has produced an uncomplicated, economical and informative account of the special theory of relativity in an elegant modern style. **P. C. W. Davies**

Drug resistance

Infectious Multiple Drug Resistance. By S. Falkow. Pp. 300. (Pion: London, July 1975.) $19.95; £7.70.

THIS is a very useful book which is in general up to date. It is the only treatise so far published which exposes, with considerable success, the practical as well as the theoretical significance of bacterial plasmids. It is a pity that the title is misleading: not only does the book deal with infectious (I prefer "transferable") drug resistance, but it presents the reader with a succinct introduction to microbial genetics and also contains a substantial amount of the relevant molecular biology. Moreover, it describes in some detail the plasmids that may contribute to virulence, at least in the potentially pathogenic *Escherichia coli* of animals and probably of man.

There is something here for everyone interested in the field: fundamental information about the structure of bacterial transfer systems; clinical data about the incidence of bacteria carrying R factors and other plasmids in man and animals; information about and speculation on the origin of the resistance and other genetic determinants and the transfer factors that can carry them, and the relationship between the two types of agent in complete transfer systems; and a useful discussion of the ecological significance of plasmid-determined resistance.

There is, however, no specific identification of references in the text. In a book of this stature, all references should be precisely indicated in the text, so that they can be related to the full references at the end of the chapter, or of the book. Chapter 4 on ecology finds itself in the genetic introduction, instead of in conjunction with chapter 10, which deals with *in vivo* transfer of R factors and their significance in public health. The terms fi^+ and fi^- are uncomfortably near synonymity with F-like and I-like throughout the book. And there was sufficient information available about the Mexican strain of chloramphenicol-resistant *Salmonella typhi* and the R factor it carried, while this book was being written, to warrant fuller description of this highly important example of the disasters that may follow the abuse of antibacterial drugs. The designation of compatibility Group O is given undeserved priority. Group B, which is identical with it, was described over a year before O.

But I am carping, and I am sure the next edition will rectify the small scattered faults. The literary style is rather breezy—not necessarily a disadvantage —and I would strongly recommend this book. **E. S. Anderson**

Environmental physiology

Environmental Physiology. By D. Bellamy, G. J. Goldsworthy, K. C. Highnam, W. Mordue and J. G. Phillips (Editor). Pp. ix+198. (Blackwell Scientific: Oxford and London, 1975.) £3.25.

THIS book attempts to present, at a fairly introductory level, a general view of physiology as it relates to animals in their natural environment; as is stated in the preface, it seeks to give expression to the conceptual link between physiology and ecology. The text is divided into three parts. The first of these introduces the animal and its environment, pointing out the main relationships between them, and goes on to consider the internal environment of the organism. As with any introductory text, basic concepts need to be outlined and it is in this first part of the book that the physiological ideas of homeostasis, active transport, osmotic pressure, nerve impulses and the like are explained.

The orientation of the material presented in the second and third parts is more ecological. Part two examines the problems and mechanisms which are associated with the organism–environment interfaces. The structure and function of the epidermis, gills and lungs, excretory organs and the alimentary tract are treated phyletically. These chapters are liberally sprinkled with examples which bring out the underlying problems facing the chosen animal types in different habitats. The third part of the book deals with specific interactions of organisms with their environment, beginning with what might at first seem to be a curious chapter to have in an essentially physiological text. The chapter is entitled 'Global diversity and animal distribution' and explains how and why climate and season vary over the earth's surface. A useful table is presented which summarises the physical features of the main biogeographical areas, their characteristic plants and animals, and the adaptations enabling these organisms to exist in each area. This chapter proves to be a valuable introduction to the third part. A series of specific problems facing animals in certain environments are discussed, with particular stress on salt and water availability, and there is also a chapter on the maintenance and function of rhythms. This is followed by a complementary chapter on the effect of seasonal rhythms on the organisms, with particular emphasis on diapause.

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The third part ends with three short chapters, one on animal colour and colour change, one on biotic relationships between organisms, and a final chapter on man's impact on the environment. The biotic relationships chapter really only whets the appetite for the increasingly important field of behaviour, and much the same sort of comment can be applied to the last chapter in which the topic of pollution is scarcely introduced. In a book of this title rather larger components of behaviour and pollution might be expected, particularly as regards the physiological effects of man's pollutants on organisms.

The general finish of the book is high although the plastic laminate of the paperback version tends to part company easily from the rest of the cover, and the book quickly becomes dog-eared in use. The overall impression is that it is a useful first and second year reading for undergraduates in both physiology and ecology and that this book may also find a place in sixth form teaching.

Bryan D. Turner

Genes, receptors, signals

The Immune System: Genes, Receptors, Signals. Edited by E. W. Sercarz, A. R Williamson, and C. F. Fox. Pp. xxiv+632. (Academic Press, New York and London, 1974.) $22.50

IT becomes increasingly obvious on reading the proceedings of the Third ICN–UCLA Symposium on Molecular Biology that an immunological event of some importance took place at Squaw Valley, California, in March of last year. Practically everyone who is anyone in the field of cellular immunology seems to have been there, searching for answers to many of the outstanding questions about the control and expression of immune responses at the molecular level.

Although there were a few reports on the structural basis of antigen (or hapten) binding sites, particularly in relation to antibody specificity and diversity, and even half a paper on the IgE system, the main emphasis was placed firmly on the genetic and cellular control of antibody synthesis.

The reader of the proceedings unfortunately misses what (from the Preface) must have been much lively and uninhibited discussion at the Workshop sessions; transcripts of which have not been included. But the published papers cover a wide range of 'up-to-the-minute' topics in the vanguard areas of immunology and should, therefore, provide informative reading for workers in many different branches of the discipline.

Inevitably many questions are left unanswered, including some key ones—such as the nature of T cell surface receptor. Nevertheless, the reader is treated to stimulating new thoughts about fascinating topics like the nature and control of antibody diversity, the relationship between the genetic control of histocompatibility antigens and immune responses, lymphocyte membrane organisation, the mechanism of development of B memory cells, and the role of T cells in immune responsiveness and tolerance. The proceedings also contain a range of valuable technical information, including descriptions of: the 'finger printing' of thymus cells by physicochemical procedures, the fractionation of lymphocytes and probing of their surface structure, the estimation of messengerRNA responsible for Ig heavy chain synthesis and the measurement of clonal development of single antibody-forming cells in culture.

The only disappointing aspect of the book is that dealing with the triggering of lymphoid cells. The treatment of mechanistic aspects of cell activation and transformation is sparse to say the least, in spite of the reference to 'receptors' and 'signals' in the title. This no doubt reflects the wide gap that still exists between immunological observation and biochemical understanding of lymphocyte stimulation processes. In view of the growing evidence, however, that immunological triggering processes have important features in common irrespective of the nature of the target cell, it is to be hoped that the next Symposium on this topic will take into consideration the valuable clues about lymphocyte function to be gleaned from the fundamental investigations now in progress on other immunologically controlled cell systems such as those involving mast cells and macrophages. **Denis R. Stanworth**

Flavonoids

The Flavonoids. By J. B. Harborne, T. J. Mabry and H. Mabry. Pp. 1,204. (Chapman and Hall: London, July 1975.) £27.50.

THE appearance of *Chemistry of the Flavonoid Compounds* edited by Geissman in 1962 and of *Naturally Occurring Oxygen Ring Compounds* by Dean in 1963 were major publishing events in the flavonoid field, and undoubtedly did much to stimulate the impressive advances which have taken place since their publication, over a decade ago. The present volume, edited by active contributors to the subject, attempts to summarise progress in the flavonoid field since Geissman's book. The former work will, however, still deserve a place on the library shelf for reference to some of the older work.

The chapters of *The Flavonoids* fall into three main groups. Firstly, four chapters concerned with isolation techniques, ultraviolet, and nuclear magnetic resonance spectroscopy, mass spectroscopy and synthetic methods. Secondly, eleven chapters concerned with the individual classes of flavonoids. Finally there are five interrelated chapters on biosynthesis, metabolism, physiology and functions, biochemical systematics, evolution.

This is a work of major importance; the many phytochemists who have been eagerly awaiting its publication will not be disappointed. It is inevitable that there will be the odd error, misquotation or missed reference; the reviewer found some pertaining to the Rosaceae, and doubtless others will find likewise within their own specialities. Overall, the editors could have exercised a greater degree of control over the general structure of each author's contribution; for example, although there may be justification for treating flavone and flavonol aglycones in separate chapters, it would be reasonable to expect the separate treatments to be along closely parallel lines—which they are not. In both, there is unnecessary overlap with material contained in the first four chapters. It seems strange that chalcones and flavanones should be dealt with in separate chapters—a somewhat unsatisfactory arrangement.

Coverage of the literature is uneven; some contributors have reviewed their topics from their beginnings, others have taken Geissman's 1962 book as their starting point and the chapter on anthocyanins takes Harborne's 1967 *Comparative Biochemistry of the Flavonoids* as its starting point in listing natural occurrences. It is not always easy to ascertain in which year the literature coverage stops, as in some chapters it is extended by an addendum at the end of the book; it does seem, however, to be intensive up to the end of 1972, with somewhat patchy and incomplete coverage of the 1973–74 literature (appearing often in both main text and addendum). Such inconsistencies are, however, difficult to avoid in such multi-author works.

The first fifteen chapters are highly factual in content, as obviously intended, but nevertheless frequently make interesting reading. The last five chapters, however, because of their more general nature, make more enjoyable, enlightening and occasionally provocative reading. One notable omission from the chapter on biosynthesis is any mention of the possible rôle of the interrelated α- and β-hydroxychalcones, chalcone epoxides, 2-hydroxyflavanones and dibenzoylmethanes in flavonoid biosynthesis; these compounds have recently aroused interest as possible biosynthetic intermediates and some informed speculations would have been welcome.

Throughout the remaining chapters there is a steadfast refusal even to admit the possibility that flavonoids (with the obvious exception of flower pigments) might be generally functionless. Suggested functions range from light screens to sex determination. Many supposed physiological functions of flavonoids might be the consequence, rather than the cause, of changes in primary metabolism. If in fact flavonoids are generally functionless, then the phylogenetic consequences are of considerable interest; this aspect has not even been considered.

The concept of 'replacement characters,' referred to a number of times (for example, page 1,066) seems of dubious validity to the reviewer. Why should betacyanin pigments replace anthocyanins, or dihydrochalcones replace arbutin? Flavones are said to replace flavonols (page 1,067) yet it would seem more reasonable to say that flavones seem in some instances to coexist with the more common flavonols, and in some instances flavones occur alone. In the reviewer's experience, when flavones appear in certain *Pyrus* species, the flavonol levels can actually be much higher than otherwise; how can this be defined as a replacement?

Criticisms apart, the book is undoubtedly a major publishing event in the field of phytochemistry. The general layout is tidy, spacious and uncluttered, and the indexes (authors, plant species and subjects) are excellent. *The Flavonoids* will serve as a major and indispensable work of reference for many years to come and can be unreservedly recommended to anyone concerned with this subject. Unfortunately the price will restrict this book to few purchasers other than libraries.

J. S. Challice

FOUR NEW
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M. F. GREAVES
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Cytogenetics is primarily concerned with the structure and function of chromosomes. This book provides an up-to-date account of recent techniques and results in this field laying particular stress on abnormalities and their medical relevance.

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obituary

Conrad Hal Waddington, CBE, FRS, the noted geneticist and distinguished scientific thinker, died on September 26 at the age of 69.

HAVING graduated from Cambridge (Sidney Sussex) in 1926 with a first class honours in geology, Professor Waddington became lecturer in zoology at Strangeways Research Laboratory, Cambridge in 1933, but later was persuaded to move to embryology in 1936. In 1947, two years after leaving Strangeways, he became Buchanan Professor of Animal Genetics in the University of Edinburgh, where he remained until his death. His practical involvements in international affairs include the presidency of the International Union of Biological Sciences from 1961–67 and the setting up and vice-presidency of the International Biological Programme (1963–66). He was also a founder member of the Pugwash Conference and the Club of Rome. His most outstanding contributions were, however, as a synthesiser of knowledge and as a populariser of science through his many books and papers. His many honours include FRS (1947), CBE (1958), foreign membership of the American Academy of Arts and Sciences, several honorary doctorates and numerous visiting lectureships and fellowships.

THE bare, formal, record of Waddington's career gives little indication that he differed much from other distinguished scientists of his generation. Yet Waddington was a very unusual man, and if his influence on science was diffuse—he established no defined school as a legacy—it was certainly very widely felt.

The transition from geology through evolutionary studies to genetics was natural, but thwarted at first by lack of employment in genetics. Experimental embryology was the next best thing. Alone, and in collaboration with the Needhams and with Jean Brachet, his ten years in Cambridge saw a number of important contributions to our knowledge of embryonic induction. Most significant were the establishment of the phenomenon in higher vertebrates, a technically formidable undertaking, and, in the second place, the discovery of inductive activity in a chemically defined but unnatural substance (methylene blue). At the same time he was theoretically active in refining the concepts of induction and, more generally, in relating the mechanics of development to the expression of genes. Many of his views (for example, in *Organisers and Genes*, 1940) are recognisable in today's thinking.

It was to be expected that he should move onto the developmental genetics of *Drosophila*, but this also heralded the beginning of a pattern that was to persist. His own experimental efforts became eclectic. He could not resist a good idea for an experiment, but he, or his students, picked up too many interesting problems for him to call any one field his own. The positive side of this breadth of sympathy was real enough. The Institute of Animal Genetics in Edinburgh became justly celebrated for work in a quite exceptional range of genetic and developmental fields. Waddington provided an umbrella under which important talents could shelter.

His own science became centred around the problems of relating genetics to adaptive evolutionary change by way of developmental processes. Waddington grappled with the constraints that developmental considerations place on the kinds of evolutionary change that are practicable in a succession of books (for example, *Strategy of the Genes*, 1957) and articles of great interest. A brief account of his scientific life (in *Towards a Theoretical Biology*, Volume 2, 1969), illustrates the enduring influence which A. N. Whitehead had on his mind, and that he was first and foremost an evolutionary biologist for whom the mechanisms of development and inheritance revealed by analysis only made final sense in an evolutionary context.

Waddington's interests extended far beyond natural science and it was characteristic that he should not regard the cultural and political movements of his time as isolated from science or it from them. His public image was first created with the wartime Pelican *The Scientific Attitude* which, although necessarily burdened by contemporary problems, still makes interesting reading.

Conversation with Waddington was always stimulating. He lacked pomposity, always had some new preoccupation to expound, and treated the work of others with the same respect whether they were eminent or unknown, personal friend or personal foe. He will be missed.

D. R. Newth

THE recent death of my old friend and collaborator C. H. Waddington left me desolated by the break with the past that it entailed. But I must say that my primary feeling was one of great distress that he should have had to go just at this time when his wisdom is so much needed for the world. There are very few people of impeccable scientific standing and acknowledged genius who yet have a concern for ethics, for human society bettered and safeguarded, and for the forms of experience other than the natural sciences, together with such a calm, humane, generous and humorous spirit. I would have wanted at least a dozen more years of his judgment and leadership. His recent lectures on 'biological engineering', and the anti-science movement of the counter-culture, at the Royal Society and the Royal Society of Arts, made a great impression.

I knew him first in the twenties when I was starting research in Cambridge, and he was a member of the University Science Club, together with Gregory Bateson and other mutual friends of those generations. The period of our association covered just exactly the decade of the thirties, for the year in which my *Chemical Embryology* was published (1931) was the same year in which Otto Mangold and others, notably Wad himself, showed that the primary neural inductor in vertebrates was stable to physicochemical denaturing agents. Out of this arose our collaboration in chemical embryology, studying what we used to like to call the 'morphogenetic hormones'—a conception still necessary today, although remarkably little progress has been made in the identification of these intermediate products of the genes.

Wad made a splendid collaborator. In intellectual grasp and analytical penetration I always felt he was gifted far beyond me. The great thing about him was that he was a man with whom it was impossible to quarrel. When one knew him only a little in those young days one might at first think him unduly reserved, even cold, with no room for the passionate at all; but this was quite a misconception, because throughout his life he enjoyed many deep friendships and attachments with people of very varying ages. At the same time he was singularly 'unflappable', a quality that stood him in good stead when in the field for geology or exploration. Always politically progressive but entirely without fanaticism, he would do everything he could to help younger people, and those who were refugees from their own lands.

Another aspect which I appreciated so much in him was his understanding of forms of experience other than that of the natural sciences, notwithstanding his fundamental and all-pervading deep rationality. In his case it was philosophy and the nature of aesthetic experience to which he was largely drawn, which might account in part for his marrying a brilliant architect; in my case it was always religion and theology, but we never had any disagreements on such matters. Besides, we came together very closely on the ground of ethics, because we were both entirely convinced that the only ultimate salvation of humanity would lie in the control of the discoveries of the natural sciences by an ethic, a morality; the latter would have to be an entirely new one, congruent with the vast increases in human knowledge and the frightening powers over Nature which humanity was acquiring and would acquire. There has been no-one in my life whom I have liked and admired more, and I can only hope that his writings and his philosophical outlook will still influence younger people for many years to come.

Joseph Needham

John Wilfred Linnett, FRS, Master of Sidney Sussex College, Cambridge, and a noted theoretical chemist, died on November 7, at the age of 62.

JACK LINNETT was born on August 3, 1913, and educated at King Henry VIII School, Coventry, and St John's College, Oxford. After holding a Research Fellowship at Balliol he was made a Fellow of Queen's College, Oxford, in 1945. He was for twenty years first a Demonstrator then a Reader in the Inorganic Chemical Laboratory in Oxford, and was appointed Professor of Physical Chemistry at Cambridge in 1965. After a period as a Professorial Fellow at Emmanuel he was elected Master of Sidney Sussex College in 1970. He served as Vice-Chancellor at Cambridge from 1973–75, and had relinquished this arduous post only five weeks before his death. He was president of the Faraday Division of the Chemical Society from 1971–73, and was to have become President of the Chemical Society in 1976. He had been a visiting professor at the Universities of Wisconsin, California, Cornell and Minnesota, and had strong ties with Malaysia and Singapore in his capacity as an External Examiner. His two books on valence theory are known to all undergraduates, and his 260 published papers covered such a variety of chemical topics that they stamp him as unique: infrared spectroscopy, combustion and flames, force constants, kinetics, catalysis, surfaces, wave mechanics. There are few aspects of physical or theoretical chemistry on which he has not left his mark. He was elected a Fellow of the Royal Society in 1955; had served the city of Oxford as a JP; and was an Honorary Fellow of Queen's and St John's Colleges, Oxford. In recent years he had become increasingly interested in the teaching of chemistry as an educational process in its own right, and devoted much time to communicating his thoughts on this topic to others. In spite of the enormous demands on his time, he was always accessible to undergraduates, research students, colleagues, staff and friends, and was willing to give advice and lend a hand whenever and wherever it was required. He died very abruptly on 7 November 1975, typically between giving a PhD oral and addressing the Royal Society, at the very height of a formidable career. It is difficult to assess how many people will be needed to fill the gap that he has left.

P. J. Wheatley

announcements

Awards

The **French Academy of Science** has awarded its main annual prize jointly to **S. Brenner** and **S. Benzer** for their achievements in the field of molecular genetics.

G. Barsky and **B. Ephrussi** have been awarded the **Paul Ehrlich Award** for their pioneering work on cell fusion.

The **1975 Albert Lasker Awards** are as follows: The **Clinical Medical Research Award** to **G. N. Hounsfield** and **W. Oldendorf**; A **Basic Medical Research Award** to **R. C. L. Guillemin** and **A. V. Schally**; Another **Basic Medical Research Award** to **F. Dixon** and **H. G. Kunkel**; The **Special Award** to **K. H. Beyer, J. M. Sprague, J. E. Baer** and **F. C. Novello**; The **Public Service Award** goes to **J. Stein.**

Appointment

K. P. Duncan has been appointed a member of the **Medical Research Council.**

Reports and publications

Other countries

Publications du Centre National pour l'Exploitation des Oceans (CNEXO). Série: Rapports Scientifiques et Techniques, No. 21: Isolement in situ d'Eau de MerNaturelle dans des Enceintes de Grand Volume: Application a l'Étude d'Une Eutrophisation Intérêt et Prospective. Par Brigette R. Berland, Daniel J. Bonin et Serge Y. Maestrini. Pp. 18. (Paris: CNEXO, 1975.) [310

Smithsonian Contributions to Zoology. No. 192: Review of Ochsenheimeriidae and the Introduction of the Cereal Stem Moth *Ochsenheimeria vacculella* into the United States (Lepidoptera: Tineoidea). By Donald R. Davis. Pp. iii + 20. No. 193: The Principal *Larrea* Bees of the Southwestern United States (Hymenoptera: Apoidea). By Paul D. Hurd, Jr., and E. Gordon Linsley. Pp. iii + 74. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [310

CERN — European Organization for Nuclear Research. CERN 75–11: Polarized Particles for Accelerator Physicists. By J. S. Bell. Pp. vii + 37. (Geneva: CERN, 1975.) [310

United States of the Interior: Geological Survey. Professional Paper 898: Slump Blocks in the Atlantic Highlands of New Jersey. By James P. Minard. Pp. iii + 24. (Washington, DC: Government Printing Office, 1974.) 95 cents. [310

Smithsonian Contributions to Zoology, No. 198: Ctenuchid Moths of *Ceramidia* Butler, *Ceramidiodes* Hampson, and the Caca Species Group of *Antichloris* Hubner. By William D. Field. Pp. iii + 45. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [610

International Institute for Applied Systems Analysis. Annual Report—IIASA '74. Pp. xiv + 42. (Laxenburg, Austria: IIASA; London: The Royal Society, 1975.) [610

Coastal Water Research Project—Annual Report for the year ended 30th June 1975. Pp. v + 211. (El Segundo, California: Southern California Coastal-Water Research Project, 1500 East Imperial Highway, 1975.) [610

United States Department of the Interior: Geological Survey. Professional Paper 837: The Logic of Geological Maps, With Reference to Their Interpretation and Use for Engineering Purposes. By David J. Varnes. Pp. iii + 48. $3.50. Professional Paper 856: Structure and Origin of the Koae Fault System, Kilauea Volcano, Hawaii. By Wendell A. Duffield. Pp. v + 12 + plate 1. (Washington, DC: Government Printing Office, 1974 and 1975.) [610

nature

December 4, 1975

21st century food needs thought — now

"Our understanding of taste physiology and of flavour technology is abysmal. If the next generation and generations beyond are going to be fed acceptably as well as adequately, we shall certainly need to do far more research and development in these fields of study than has been contemplated hitherto . . . in my opinion the survival of our western civilisation is going to depend on a new food revolution—by which I mean a revolution in our methods of processing food, in increasing the shelf-life of our food, and in improving our methods of distribution. In practically all advanced countries agricultural research still attracts vastly greater resources than does food research. This imbalance will surely have to change."

Those words of Lord Zuckerman at the British Nutrition Foundation's Annual Lunch brought loud 'hear-hears', banging on tables and tapping of coffee-cups. They obviously struck a responsive chord amongst the mixed audience of food industrialists, academics and civil servants, but that is hardly a surprise since more research and development means more jobs and more government money. Is there, however, more to this than the conventional response of any interest group?

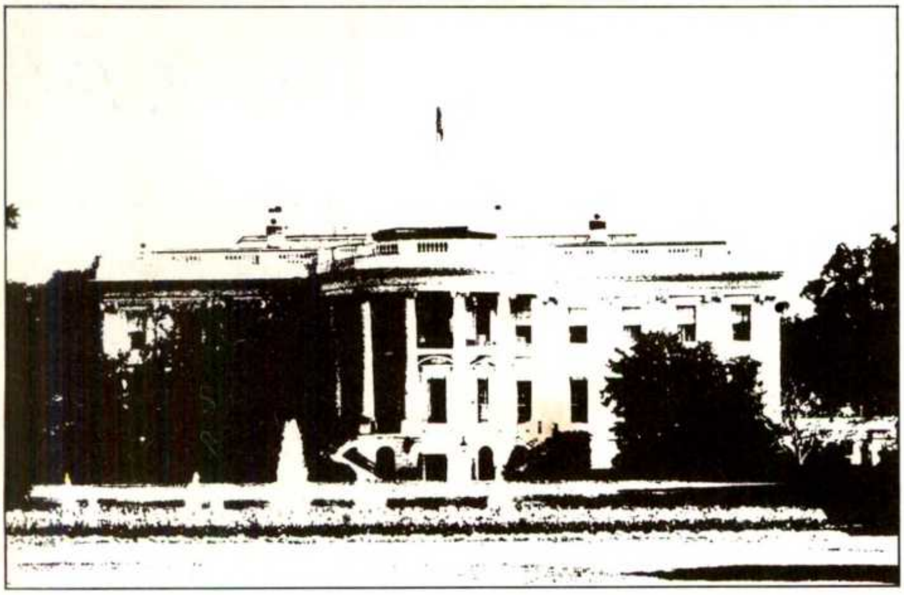
The more one looks at the sciences and technologies connected with food, the more one realises how ignorant we are and how unscientific our approach is to food. This is, strangely, not a statement that has to be dragged out of a profession in the face of massive public relations effort to convince one that all is well and that there are many fine minds at work; it is one that is heard regularly and almost despairingly from the practitioners themselves. It seems that it is often almost impossible to attract bright students into the field; mainstream academic thinking (with the encouragement of the Nobel committee) must take the blame for much of that. It is praiseworthy to have developed a new and better strain of rice, or to have worked out at the molecular level how something works. But to have developed a new means of preserving food, or a way to persuade the average citizen to consume textured vegetable protein; these are mere technical advances which can presumably be done by people with lesser qualifications.

Another disincentive to the bright youngster is the evidence, all around us, that we are better fed than ever before. In Britain there is plenty of variety—the nutritionist's first requirement—and the calorie value of food 'moving into consumption' is 10 % higher per capita than it was in 1945 and a full third higher than the national average requirement, permitting us to be more wasteful. Nutritional diseases are rarer (except for dental caries)—surely everything challenging has been done already.

The same sort of things were being said in the 1960s about energy, and now we are busy looking for alternative sources and ways to persuade the public to be energy-conscious. Food has already sounded one early warning of future crisis; surely now is the time to be doing intensive research on food options including acceptability, for the 1980s and beyond. There is no sign that anything like enough is being done.

To pursue the analogy with energy, one of the weaknesses of the present "save it" campaign is that it is trying to put into reverse the "use it" advertising so common until recently alongside which the government took no discernible action. The lesson for food must be that whilst there is still great variety and whilst quantity is no problem, there must be a growing governmental involvement in providing information and education. If launched now, such a campaign need have no negative overtones but could instead encourage the great eating public to be participators in a genuine experiment to evolve new, acceptable foods. Adult tastes can change (or rather, grow)—the recent proliferation of take-away Indian and Chinese restaurants is testimony to that—but the increase of nutritional awareness amongst children is probably a higher priority. The daily meal at school provides an excellent focal point for the discussion of nutritional matters; already a few schools permit their pupils to help plan weekly menus. This sort of practical involvement is an ideal form of education.

We have only two options available in thinking about future food supplies. The first is to hope that the crunch will not come in our lifetime. The second is to invest heavily in research, development and education so that if the crunch does come we can confront it in a prepared and mature way. □



Wanted: new science policy

In the United States, as in the rest of the developed world, the twin-headed international monster of simultaneous inflation and recession has, by threatening an era of no growth, loosened the financial adhesive that has until now made technology and progress virtually synonymous. It may be time for innovation itself to take a new form. Wil Lepkowski reports.

FOUR years ago, as President Nixon was preparing schemes for waging his re-election, one of his aides took the notion that "technology" might be a viable campaign tool for harnessing what was left of a sympathetic technical community. Out of the woodwork flew William M. Magruder (no relation to Watergate's Jeb S. Magruder), who until then had been the government's supersonic transport lobbyist. History records that Magruder, aeronautical engineer and former test pilot, failed in his attempt to sell Congress on the merits of supersonic passenger flight. But Nixon gave him a second chance. He appointed him to assemble a "New Technological Opportunities Programme".

That failed, too. Nixon's own Office of Management and Budget allowed Magruder to huff and puff in a vast interagency search for the finest, economically most relevant set of programmes. But it saw that the best ideas were already being funded and the "new opportunities" were simply unfunded dreams taken from dusty file cabinets. Nixon subsequently unfurled a platitudinous science and technology message in March 1972 (less than a year before he wiped out the White House science advisory apparatus). Magruder was given a large office to rethink his goals, and the myth that crewcut, phallic technology was the answer to America's challenge of the 1970s sputtered to an end.

The economics of America weren't good then, and heaven knows they are much worse now. Yet curiously, Nixon's successor, Gerald Ford, wheels out no latter day Magruder. William M.'s perceptions, like Jeb S.'s ethical compass, were off by something like 135° in a value system that continued to stress large scale, power hungry, complex systems at the expense of the sophisticated but smaller scale technologies that seem needed today. The curiosity is that Ford, who, in a man by the name of William Goldwin has an intellectual advisor to flag him on critical future issues, hasn't uttered the slightest hint that maybe a new look at technological innovation and the scientific base needed to sustain it might be part of the way to solve the country's potentially disastrous outlook.

Technical Washington is tired, dejected, and cynical—a virtual burnt-out case. "There seems to be a malaise that has set in," says a National Academy of Sciences staff member, "and it has deepened. Members of the committees I work with seem to feel that nothing they do matters".

Big technology, however, does churn forward, chiefly in the form of energy projects under the Energy Research and Development Administration. And in the field of health services, technological impersonalisation in the name of productivity holds policy sway, in spite of the Department of Health, Education, and Welfare's new dedication to prevention as the programme perspective for the next 10 years. In both fields, central to national welfare, costs and consumer dissatisfaction soar. One Washington source observes that present leaders continue to wear the same perceptual filters that brought the country to the its present burnt-out state. He says we need a new set.

The new set could come in the form of a thoroughly new look at innovation for an era of no growth. Merely beginning the exercise would be monumental in itself, for the assumption is that economic growth must resume if any innovation can happen. More to the economic point, conventional wisdom has it that the economy simply must grow if American industry is to raise the capital to build the machines to provide the jobs to prevent social unrest. Industry needs around $4.5 million million in new capital by 1985 to do little more than stay even. Few of the best banking heads know where even half that will come from.

There is trouble ahead, and if there was ever a time for the generation of new ideas it is now. The maddening fact, however, is the almost complete lack of debate over technological and scientific innovation. Almost . . . for there is a twinkle.

The twinkle is Princeton University economist Robert Gilpin, who has produced a report for the economic growth subcommittee of the joint economic committee. Gilpin's report lacks the substance that could come from discussing 'things' in the form of the ideas and inventions that offer a way out of the mess. But he gives broad policy hints that say we should at least be nimble.

> ❛The maddening fact is the almost complete lack of debate over technological and scientific innovation❜

"The manner in which we manage the so-called energy crisis will be very instructive with respect to our capacity to rejuvenate our economy in an intelligent way," he says. "On the one hand, the resource, environmental, and related problems affecting our economy may very well be the functional equivalent of catastrophe. They can and could force the long-term rebuilding of our technological-industrial infrastructure. New demands and needs have been created which, with proper incentives, could lead to the innovation of new industries and technologies which in turn will generate new technology-intensive exports.

"On the other hand, in our anxiety to find a quick and short term solution to our energy and related problems we could, through government subsidisation of large and commercially inefficient technologies, harm our economy. In pursuit of energy independence and security of supply, we may lock our economy into a high, noncompetitive price of energy." He might also have added health and welfare costs.

Gilpin calls for more planning of America's future. But his caution is that economic planning in the past has been justified usually in terms of protecting rather than rejuvenating industry. What seems to be needed is a thorough examination of the scientific enterprise and the ways its own mode of operation is governed by outmoded economic and industrial priorities.

“The point is that the issue of innovation during a time of sweeping economic change is crucial and necessary, but tricky”

The point is that the issue of innovation during a time of sweeping economic change—and the anxiety that accompanies it—is crucial and necessary, but tricky. It calls for breaking through to new perceptions without falling into the traps of faddism and technological cynicism.

All over the country, small groups of scientists, technologists, artists, children and plain people are forming communities built on small scale self-sufficiency technology. To them, this is innovation. And having given up on their government, they are going their own way with the old string and sealing wax approach. Except they understand optics and electronics and mechanical inventions as well. Strangely, neither Ford nor the Energy Research and Development Administration, nor the Department of Housing and Urban Development, nor the Office of Technology Assessment in Congress are doing anything to tap this potential rescue army.

Meanwhile, its troops buzz away constructing their beehives according to the laws governing the economics of nature. Others are designing new health maintenance systems built on the premise that the body tends to heal itself and can be promoted to do so without heavy resort to medications. And no one up high notices them for the innovations they could represent. The worst offender is none other than the National Science Foundation, whose mission is to search out and fertilise the innovator.

All of this is understandable, however. The 'new innovators' aren't always the most patient thinkers, and many are outright anarchists. One is Karl Hess, Barry Goldwater's former speechwriter, who in one motion evolved from conservative to radical in his politics, now refuses to pay any income tax—claiming to own nothing—and is working on a neighbourhood self-sufficiency project in a Washington ghetto. Still, Hess and hundreds of other such community leaders may have something if neighbourhoods in the middle class suddenly find their natural gas supply at an end and jobs not available.

Perhaps one of the problems is that Ford and his advisers refuse to consider the worst of all possible futures (such as the collapse of the central banks) when technology for self-sufficiency would be the policy.

A policy for the worst possible future would create the lean and elegant technologies that just could turn the society back to a sense of benignity and small scale. There are some harsh economics truths in the air at present that indicate the advisability of a 'new science policy'. The financial markets are in virtual turmoil, owing to the barely averted collapse of New York City and, with it, New York State. The ripple effect through the entire socio-economic structure of the country is too vast to contemplate if the country cannot pay its bills to itself. Everything stops, except the 'life urge' itself—and that is the ultimate of any economic or science policy; human needs. There is no fooling around about such matters. But, curiously, leaders are ignoring them. ☐

Ford: should he consider a technology for self-sufficiency?

Behind any cod peace

KENNETH MELLANBY

FISH, as all admirers of the late P. G. Wodehouse's Bertie Wooster well know, is a food which nourishes the brain. Before he tackled any particularly taxing problem, Jeeves was urged to consume a dish of cod. It is to be hoped that all those trying to solve the problems of fishing in Iceland's territorial waters will keep their mental faculties in tip-top order by themselves eating some of the fish which are the cause of the trouble!

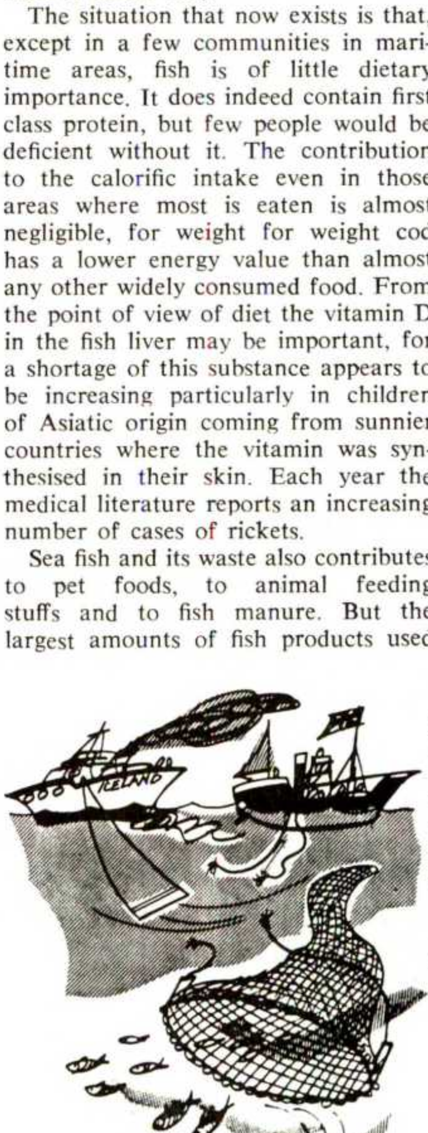
The problem is one of marine conservation. Catches of cod and other fish off the coast of Iceland have been falling and biologists believe that unless the tonnage caught is substantially reduced, the industry will suffer permanent damage. This would be a major disaster to Iceland. As far as Britain is concerned, the livelihood of a proportion of our 17,000 fishermen, and of the east coast ports from which they operate, would be affected, but our national economy is hardly at stake. Nevertheless we would clearly benefit if we could continue to share in a sustainable yield, and we could make long term plans accordingly.

The importance of fish in our diet has often been overestimated. When there was a general belief in a global "protein gap" there was a hope that the oceans would make a substantial contribution to offset shortages of essential elements from crops and animals grown on dry land. The high levels of first class proteins in fish made them particularly valuable in this connection. A great deal of research has been done to try to maintain and increase fish stocks, and to develop fish farming in both marine and freshwater habitats.

Nevertheless, in most western countries, fish consumption has steadily decreased. During the nineteen thirties people in Britain ate on an average some 30 pounds of fish annually, and it is reckoned that over 70 per cent of white fish went to fish and chip shops to provide a cheap and nutritious meal for the poorer sections of the population. Today we only eat about 12 pounds a year—some four ounces a week. Fish and chip shops have disappeared from many towns, or deal mainly in other foods. Many towns no longer support a "real" wet fish shop. The main source of fish for most of our population is the frozen food cabinet in the supermarket and the most popular dish the fish finger (which, incidentally, may have a higher content of batter and other extraneous matter than of actual fish).

The situation that now exists is that, except in a few communities in maritime areas, fish is of little dietary importance. It does indeed contain first class protein, but few people would be deficient without it. The contribution to the calorific intake even in those areas where most is eaten is almost negligible, for weight for weight cod has a lower energy value than almost any other widely consumed food. From the point of view of diet the vitamin D in the fish liver may be important, for a shortage of this substance appears to be increasing particularly in children of Asiatic origin coming from sunnier countries where the vitamin was synthesised in their skin. Each year the medical literature reports an increasing number of cases of rickets.

Sea fish and its waste also contributes to pet foods, to animal feeding stuffs and to fish manure. But the largest amounts of fish products used in this way include such substances as the Peruvian anchovy, rather than the catches of our British-based fleet.



"For us it could be 'Peace in Our Time'"

Cartoon: Mahood, Financial Times

Notwithstanding the comparative unimportance of fish to our nutrition, the industry is an important one, and this should be the basis of negotiations with the Icelandic government, and of international discussions shortly to take place on the Law of the Sea and of the rights of maritime and fishing nations in the water off their coasts. It is to be hoped that "red herrings" such as the protein levels in the diets of Western nations will not be allowed to obscure the issues. Eating fish gives pleasure to many, and makes their diet less monotonous. Our fishermen have hard and dangerous lives, and deserve to be adequately rewarded. Research has taught us a lot about population dynamics and about how fish stocks can be conserved, though some of the recent changes in numbers and distribution—for instance the disappearance of herring from many traditional fishing grounds—are not fully understood.

It looks as if considerable restraint in fishing will be needed in breeding areas such as that around Iceland, and that increased catches of species which the housewife presently finds unacceptable may be needed if consumption is not to fall even further. Fish farming in sea lochs and freshwater will no doubt increase, but it is unlikely that fish will ever form more than a tiny fraction of the world's food supply. The growing world population will need to be fed on the increased productivity of terrestrial rather than of marine crops and animals.

As for the worry about the energy used in modern farming, where the crop produces fewer calories than are expended in producing it, this applies even more to fisheries. The fishermen themselves consume about a quarter of the calories of their catch, their boats use many times as much energy, and this total is further increased when that needed to build the ships and manufacture the fishing gear is included.

● Mr Jens Evensen, Norway's minister for matters relating to the law of the sea, has suggested a law empowering the Norwegian Government to introduce a 200-mile economic zone off the country's coasts. The suggestion, which came in a report being debated this week by the Norwegian parliament, is designed to prepare in advance the legislation that would be necessary when a solution is found to the problem of extending Norway's fishing limits. It allows for the introduction of the wider non-trawling zone gradually, and also for narrower limits that relate to types of catches and gear at different times of the year. □

international news

Appointment to Soviet Academy threatens autonomy

from Vera Rich

THE election of Academician Anatolii Petrovich Aleksandrov as President of the Soviet Academy of Sciences, to replace the interim President appointed last May, Academician Vladimir A. Kotel'nikov, took place in an atmosphere which may well indicate a greater degree of governmental supervision of the Academy's work in the future.

It is significant that in the election of the last two presidents, Nesmeyanov in 1951 and Keldysh in 1961, it was an academician who proposed their candidature to the general meeting of the Academy. For on this occasion, following a brief statement from Kotel'nikov that the Presidium of the Academy had recommended Aleksandrov for the post of President, the main speech of proposal was made, not by a member of the Academy, but by the Secretary of the Central Committee of the Communist Party, M. A. Suslov. Furthermore, in addition to proposing Aleksandrov, who is also a member of the Central Committee, Suslov took the opportunity to outline what may well prove to be the official science policy of the Party for the next five years.

While stressing that "the Party gives foremost significance to fundamental research", he also stressed the importance of fundamental research for "all aspects of human activity, increasing the efficiency of social production, the social consequences of the scientific and technological revolution" and laying "new main lines of scientific and technical progress." Quoting Brezhnev's speech at the recent Jubilee celebrations of the Academy, he reiterated that "the Party expects from scientists ever deeper and bolder investigations into new processes and phenomena, an active contribution to the work of scientific and technical progress, a thoughtful analysis of problems which arise, and responsible recommendations on the best means of solving them in the interests of strengthening the power of the country, improving the life of the people, in the interest of the building of Communism".

Drawing attention to the forthcoming 25th Conference of the Party, which will approve the "Theses" of the next five year plan, Suslov said that this would be a plan of "quality and productivity", in which science would be expected to play its part in applying the results of basic research to technological practice and emphasis would be put on a closer connection between research and material production. It would therefore be necessary, he asserted, to improve the control of all academic establishments throughout the Soviet Union, so as to establish a "united system, working clearly and in coordination". This system would include the Academy itself, together with its various new scientific centres and the Academies of the Union Rupublics. Attention must be given, he added, to those Institutes of the Academy where there are still "insufficiencies and unresolved problems", in order to utilize fully the "enormous scientific potential" of the country. A greater return is thus expected on government investment in a science.

This is, of course, a politician's speech, and perhaps would merit less attention had it been delivered simply as an address from the Party Secretary to the meeting of the Academy. However, by making such a statement in a speech proposing the new President of the Academy, it might also be construed as presaging a greater involvement of the Party in the running of the Academy. It is noteworthy in this respect that, while Keldysh was elected to the Central Committee of the CPSU in 1961, the same year as he took office as President of the Academy, Aleksandrov has already been a member of the Central Committee for nine years, and his involvement in the higher echelons of Party life must accordingly be considerably greater.

Aleksandrov is, of course, well-fitted scientifically for the prestigious post of President of the Academy. His scientific interests are wide ranging, and range from high-polymer compounds to nuclear energy. In the 1930s he was part of Ioffe's nuclear research team in Leningrad, and later worked with Kurchatov, whom he succeeded in 1960 as director of the Kurchatov Physics Institute of the Academy. He has won both the Lenin and Stalin (now State) Prizes for science and holds a number of Soviet awards including the Order of Lenin, which he has received seven times. He is well-known internationally for his participation in the Geneva Conference on the peaceful uses of atomic energy in 1958 and the sixth Pugwash Conference. He also has considerable experience in practical applications of fundamental research and in the coordination of research activities, and is a skilled scientific administrator. In effect, on the basis of his curriculum vitae, and taking into account the trend of Party policy for science as outlined in Suslov's speech, he would seem an ideal candidate.

There is, however, one major factor against him. At 72 years of age he is older than both Kotel'nikov and Keldysh. He can hardly hope for a long term of office, and, it would seem, must be regarded as effectively another interim President. The greatest significance of his election for the future of the Academy may well be the manner of it. The Academy has always attempted to maintain an autonomous status in Soviet life. The close participation of Party Secretary Suslov in the election of the new President may well presage a situation in which its independence will in the future be considerably more circumscribed. ☐

Nuclear exporters agree safeguards

FOLLOWING a series of secret meetings held in London since last April, senior officials representing the seven industrial nations which supply most of the world's exports of nuclear materials have reportedly reached agreement on means of preventing the adaptation of nuclear technology to military ends by other countries.

The Nuclear Suppliers Group, or Group of Seven, came together following India's explosion of a nuclear device based on imported Canadian technology. It consists of the UK, the USA, the USSR, France, West Germany, Canada and Japan. The notable exception—China—is thought not to be ready to export nuclear technology at the present time.

The agreement seeks to establish more stringent safeguards governing the ways in which nuclear material, equipment and expertise is used by the Group's customers. It does not apparently cover the export of nuclear reactors, focusing rather on uranium enrichment, the reprocessing of nuclear fuel and the production of heavy water. The precise details remain a secret, however, though the nuclear countries may want explicit undertakings from their customers that they will not use nuclear material supplied for civil purposes to produce nuclear weapons.

It is understood that each party to the agreement will simply inform the others of its intentions to observe the agreed safeguards, which are expected to be presented at the meeting in Vienna early next year of the Board of Governors of the International Atomic Energy Agency (IAEA).

There may also be a proposal at the meeting to make more countries subscribe to the IAEA's existing safeguards. At present they apply only to countries which are signatories to the nuclear Non-proliferation Treaty, but the hope is that countries like Brazil, Egypt, Israel, India and Pakistan—all of which have not signed the treaty—might state their intentions more specifically if they are given the clear option of agreeing to the safeguards.

It is also hoped that other countries with the potential to become exporters, like Sweden, Holland and South Africa, will adopt the new code just agreed by the Group of Seven. But whatever the outcome, a central problem remains: it is not certain that a particular nuclear experiment—such as an underground explosion—can be established, to the satisfaction of the group or anyone else, as essential to the development of nuclear energy, rather than a preliminary step to the manufacture of a nuclear weapon. Which, given India's case, is where the Nuclear Suppliers Group came in. ☐

Moves to keep the dragon

IT now seems that the UK-based OECD Dragon High Temperature Reactor Project, the continued existence of which has been in doubt for the past few months, will carry on for at least nine months. Compromise proposals made last week in Brussels, but subject to clarification in further meetings this week, mean that there will be time for the Dragon Board of Management to sort out the bones of an agreement with the US Energy Research and Development Administration (ERDA), which has expressed interest in using the Dragon reactor at Winfrith, Dorset.

The villain of the piece is widely acknowledged to be the UK. The Department of Energy, presumably egged on by the Treasury, has in the recent past expressed complete reluctance to the idea of contributing any more money to a project which it now sees as well outside the mainstream of British reactor development—at present centred on the 'Steamer' pressure-tube reactor, and, in the longer term, the Fast Breeder Reactor.

Back in September, formal steps had to be taken to run the project down because there seemed no prospect of agreement about future funding. Then the UK relented a little by agreeing to a stay of execution until the end of March next year. Now West Germany has apparently convinced the EEC to see that deadline extended by a further six months.

The EEC is closely involved because Euratom is one of the participants in the project; smaller participating countries like Austria, Switzerland and Sweden have said that they are happy to go along with the German proposal, so Brussels calls the tune. What remains to be clarified this week is the precise meaning of a UK suggestion to the effect that satisfactory financial arrangements must be on the horizon by the end of March.

Dr L. R. Shepherd, chief executive of the Dragon project, said last week that he hoped the UK would adopt a reasonable approach to the problems, and not stick too rigidly to its argument that Dragon is no longer of any direct use to the UK's nuclear programme. Whatever mistakes the UK may have made in its Advanced Gas-cooled Reactor programme, he said, it still has unsurpassed experience of gas-cooled reactors, and it would be folly to opt out at a time when the ERDA is looking favourably on collaboration.

Quite apart from that, the United Kingdom Atomic Energy Authority does quite nicely out of Dragon because, even now, it gets back from the project more money in payment for services and so on at its Winfrith site than the UK pays out to be a member of the Dragon project. If ERDA makes a contribution, and the UK pays less as a consequence, the situation will look even more attractive.

That said, however, it needs to be remembered that ERDA is rather unsure of its position at the moment because General Atomic in the United States has been busily losing all its commercial orders for high temperature reactors during the past few months—partly because electricity utilities in the USA are short of money and partly because the anti-nuclear lobby has been so conspicuously successful in getting its message across. ☐

Indians ask for more nuclear power

Jullundur

INDIA has yet to display the trends apparent in small-town communities in the United States which react strongly to moves to establish nuclear power plants in their areas out of concern over possible radiation hazards. On the sub-continent nuclear energy continues to rate highly. Most people consider it prestigious and modern.

With the number of reactors in India still relatively small, most people are both unaware and unconcerned about the hazardous aspects of nuclear energy. In fact, one of the most persistent gripes that eastern states have had is that the Atomic Energy Commission (AEC) has time and again refused to concede their 'just' demand for a nuclear power station in their regions. The state of West Bengal has actually made it a cause, leading a vigorous battle on the issue.

The AEC's long-held view is that nuclear power generation in the eastern region, where most of the country's coal mines are located, is neither necessary nor economically viable. An AEC study concluded that nuclear power within 800 km of a coal pit-head would be uneconomical, and two further detailed studies carried out by the Central Planning Commission suggested that there was no need for a nuclear power station in the eastern region.

Not to be deterred, the West Bengal government pressed its demand. Then came the oil crisis, which pushed up all prices, including that of coal. Freight rates also went up, making coal haulage more expensive, and the West Bengal government pressed its claim for nuclear power even more vigorously.

The central government may finally have to yield to regional pressure. An official of the West Bengal government has claimed that studies carried out by the AEC in 1973–74 had reduced the break-even radius for nuclear power to only 300 km, with the "changed economics of coal", and the new studies would further reduce the radius to less than 150km.

If this happens, it would help "justify" the West Bengal demand for a nuclear power station, because the site the state had proposed for the purpose is situated some 250 km from the Asansol coal mines. Although West Bengal is an industrially advanced state by comparison with others which already have nuclear power, it remains debatable whether there is much to justify its continued insistence on having a nuclear power station—whatever the economics of coal. ☐

Letter from Sweden

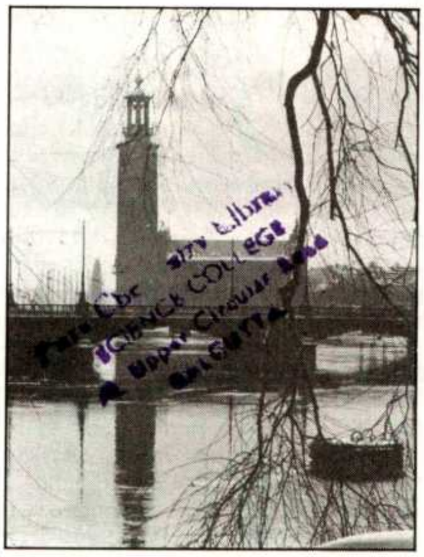
from Wendy Barnaby, Stockholm

When Myron B. Kratzer, Acting Assistant Secretary of State for Oceans and International Environmental and Scientific Affairs in the USA, spoke about international cooperation in nuclear energy in Stockholm recently, last week's agreement in London between the world's seven major nuclear suppliers had not been concluded. That was probably just as well. His speech was unrealistically optimistic as it was, without also voicing the official claims that the London agreement will probably precipitate: that the risk of the proliferation of nuclear weapons has been minimised.

Mr Kratzer was addressing the Atomic Industrial Forum on the Nuclear Fuel Cycle. His assessment that the International Atomic Energy Agency safeguards and the Nuclear Non-proliferation Treaty (NPT) have served their purpose well in stopping the proliferation of nuclear weapons can hardly be taken seriously in view of the disastrous results of last May's NPT review conference. Those signatories who attended were unable to agree on any of the major measures proposed to help stop proliferation. In general, the strength of the NPT has been sapped by the failure of the nuclear-weapon parties to the Treaty to fulfil the few obligations it demands of them. And, of course, France and China—as well as many near-nuclear states—have not even signed it.

To maintain, with Mr Kratzer, that "the record of the past 20 years on international nuclear cooperation gives considerable basis for optimism that nuclear development . . . can take place without contributing to further proliferation" is to ignore the factors which will make the next 20 years different from the last: a level of technical sophistication which will enable many states to manufacture their own nuclear weapons, the really dramatic spread of nuclear technology, and the conviction of the non-nuclear states—fostered by the behaviour of those states possessing nuclear weapons—that such weapons are desirable for national security.

● Sweden's new International Energy Institute, scheduled to open at the beginning of 1977, might do well to consider some of the areas recommended for research by a recent gathering of international energy-oriented academics near the southern city of Gothenburg. The meeting was arranged by the Royal Swedish Academy of Science in conjunction with the Natural Sciences Research Council and the Atomic Research Council.



SCIENCE COLLEGE Upper Circular Road CALCUTTA

The research suggestions concerning energy in less developed countries sounded the more convincing for the inclusion in the group of the Sudanese Minister of Agriculture, Dr Hussein Idris. Pointing out that an injection of energy into an almost feudal society can widen the gap between rich and poor, the group urged that research take into account the social as well as the biological aspects of ways of solving the immediate problems: the production of food and raw materials.

Other proposals to emerge from the meeting suggested a look at alternative ways of using energy, especially in industry. The academics called for the development of a clean method of burning coal, and advocated a study of the problems of the breeder reactor.

The group studying the side effects of energy production and use suggested that cost–risk–benefit analyses of different energy sources be undertaken, and urged that the side effects of each source be identified and used as resources in some other activity. This group produced a very wide-ranging and valuable list of research areas, including the effect of energy production and use on the Earth's climate, the ways in which scarcity of energy could lead to conflict and the effect of fast-changing technology on social relations. Also proposed were studies of the biologically disturbing effects of different energy systems on different levels of organisation (cell, organism, experimental animals, ecological systems) to determine the mechanisms whereby energy systems cause damage to health; analyses of the effects of energy use on man's health, using information from registers of cancers and birth defects; an examination of energy wastage; and an assessment of the harmful effects of energy production and use in nature.

● Changes in the climate of northern Europe have been blamed for the decrease in the number of eels in Swedish waters. Professor Gunnar Svärdson, working at a freshwater laboratory near Stockholm, has concluded that the usually cited local villains—pollution of the Baltic and overfishing—are not to blame.

Between 1964 and 1969, the average annual catch of eels in Sweden was 1,380 tons. Between 1969 and 1973, this figure dropped to 955 tons. The eels come to Scandinavia through the North Sea from the Atlantic, and on their arrival in Sweden are counted at collection stations and distributed throughout various rivers. As the 20 stations whose records are available all agree that the numbers arriving have decreased, Professor Svärdson turned his attention to the factors stopping their arrival, rather than the conditions they find when they reach Swedish waters.

One such factor is the temperature of the North Sea, which, with the exception of the past three years, has been falling steadily over the past three-and-a-half decades. Although the drop is not measurable in terms of degrees, it has nevertheless significantly decreased the amount of plant and animal plankton, an important food for eel larvae and young eels. At the same time, Arctic fish such as cod have increased their visits to the North Sea—and cod love to eat eel larvae. Meeting less food and more enemies, fewer eels survive their passage across the North Sea.

Like the water temperature, the pattern of prevailing winds has also altered. The westerly winds which sweep over the British Isles and help to point the eels in the direction of Scandinavia blow on only 80 days a year now, instead of 110 as used to be the case. Most of the newly calm days, moreover, fall just at the time that the eels are north of Scotland and would normally be turning towards Sweden.

● In the face of public and institutional protests, the mining company LKAB has withdrawn its controversial application to extract uranium from a 15-km^2 area around South Billigen (see *Nature*, **257**, 733; 1975). It will make another application next year, after revising its plans to take more account of the need for environmental protection.

Resuscitation for advanced ground transport?

THOSE in the UK engaged on research into advanced ground transport, and in particular transport by magnetic levitation and propulsion ("maglev"), suffered a sorely-felt setback two years ago when the Conservative government decided not to continue its support for the work of Tracked Hovercraft Ltd.

If they needed a reminder of how unlucky they were by comparison with their contemporaries working years earlier on supersonic air transport—who were more fortunate in being allowed the indulgence of the "white heat of technology" era, when economic obstacles were less obvious—it came last week in a report from the Science Research Council (SRC).

The report records the reasons why a panel established by the SRC's Engineering Board rejected a request from a group of universities for support for a programme of research using the hovertrain test-track facilities at Earith, near Cambridge. The application followed a recommendation from the House of Commons Select Committee on Science and Technology that the track should be maintained as a focus for research work.

The report's main conclusion is hardly encouraging. The prospects for the adoption of maglev techniques for the "slowly evolving" urban developments, or for inter-city ground transport at speeds in excess of those of the Advanced Passenger Train (APT), are, it says, "at best highly uncertain". One important reason for this, it adds, is "the lack of knowledge of the technology, and therefore of the likely cost and performance obtainable . . . when seen in the context of the world economic situation".

But the report's real emphasis is on the absence of an adequate body of knowledge, which was underlined by the "lack of success" of large-scale demonstrations. Moreover, "our comprehension of the theory, design and performance of the various forms of linear motor is inadequate".

The report's recommendations, however, offer the scientists more hope. The SRC, it suggests, should encourage analytical studies of linear motors and magnetic suspension systems, experimental verifications of the analyses and, significantly, assessments of the economic implications and safety requirements. And it also proposes that the panel be reconstituted to stimulate research, review the technology and examine the case for a rotation-type test facility, for which a financial commitment of £2.5 million over five years is tentatively indicated.

Responses to the report from universities, government departments and such concerned industries as British Rail, all of whom were represented on the panel, are now awaited. Grounds for optimism must be few and far between, given the deterioration in the country's (and British Rail's) economic position since the project was dropped amid wide publicity. □

Improving outlook for tidal energy

by Allan Piper

THE slow march towards a fuller exploitation of some of Britain's more unorthodox energy resources advanced another step last week when a proposal for harnessing tidal power in the Severn Estuary received a generally favourable hearing by the House of Commons Select Committee on Science and Technology. The hearing gave leading proponents of the scheme a valuable opportunity to redress a balance that tipped against them following the recent publications of an adverse report by the Central Electricity Generating Board (CEGB), and it seems likely that the committee will have been sufficiently convinced of the project's viability to recommend that it becomes the subject of a full-scale feasibility study.

Meeting in Bristol to take evidence on the Severn Estuary scheme as part of a wider investigation into novel energy sources, the committee heard that the CEGB's main reservations about the cost of a tidal barrage were based on outdated and erroneous assumptions. The main criticism came from Dr Tom Shaw of Bristol University's Department of Civil Engineering, who challenged the estimate that the capital cost involved in the project would be twice that needed to produce an equivalent energy supply from nuclear sources using the American Light Water Reactor (LWR). He claimed that the estimate was no longer valid because recent independent estimates had put the figure at nearly double that quoted by the CEGB. The calculations have in any case been overtaken by Britain's decision not to use the LWR.

Dr Shaw, who has been involved with the project for 10 years, also contested the CEGB's suggestion that the construction of the barrage would consume more energy than would ultimately be produced. He told the committee that once the project was completed, at an estimated present day cost of around £1,500 millions, its pumped storage facility would provide for the continuous generation of electricity for little or no further capital outlay.

CEGB representatives at the hearing did not dispute Dr Shaw's claims, and the board has indicated its willingness "to cooperate in full" over the future of the scheme. Nonetheless, their apparent opposition to the project so far raises the question of whether the control of any electricity it may ultimately produce need necessarily rest with them rather than with a specially created alternative authority.

The idea of throwing a barrage across the Severn Estuary, which provides an estimated two-thirds of Britain's exploitable tidal energy, was first suggested seriously as long ago as 1924. The latest scheme involves the construction of an h-shaped barrage, the main part of which would span the Estuary from Weston-super-Mare to Cardiff. The pumped storage scheme would operate using the smaller barrier across Bridgewater Bay. Once operative it could produce about 30,000 MkWh a year, which is about 10% of the present electricity demand met by the CEGB and about the same as the anticipated output from nuclear sources.

Though the figures and Dr Shaw's evidence may have gone some way towards convincing the Select Committee of the scheme's viability, several reservations remain to be dispelled before it can be given the go-ahead. Not least among these are the problems involved in the construction of the barrage, and its likely effect on the estuarine environment and local shipping movement. Last week's hearing included evidence from local civil engineering consultants, the Severn Valley Water Authority and the Port of Bristol Authority.

Though the attitudes of the SVWA and PBA are apparently not wholly favourable towards the scheme, the corresponding authorities on the Welsh side of the estuary may be less antagonistic. The artificial high tide created by the barrage, for instance, would benefit the port of Cardiff, because for the first time tankers with displacements of up to 150,000 tonnes would be able to approach to within a few hundred yards of the quayside.

Before the Select Committee closes its investigation into the project—a report is unlikely to appear much before the middle of next year—it will call the Secretary of State for Energy, Tony Benn, to give evidence. His ministry has presented him with an unfavourable report on the scheme, and the committee will be interested to learn why the project is not considered worthy of a feasibility study.

Whatever the details of the committee's eventual recommendations, supporters of the scheme should be encouraged by the unofficial attitude of some of its members. Following last week's hearing Chairman Arthur Palmer openly admitted on British television that he was optimistic that the first part of the project could be completed and producing electricity within ten years. □

New president for Royal Society

LORD Todd has been elected President of the Royal Society for the next five years; he succeeds Sir Alan Hodgkin, president since 1970. The new president is 68, and is not entirely new to the office—his father-in-law, Sir Henry Dale, was president of the society from 1940–45.

The long list of positions he has held and honours he has received includes periods of research or lecturing at Glasgow, Frankfurt, Oxford, Edinburgh, London, Caltech, Manchester, Chicago, Sydney, MIT, and (since 1944) Cambridge. It also includes the Nobel Prize for Chemistry in 1957. From 1952–64, he was Chairman of the Advisory Council on Scientific Policy, and he was President of the International Union of Pure and Applied Chemistry from 1963 to 1965.

Sir Alan, in his retiring address, was critical of the government for its failure to appoint a chief scientific adviser to the Cabinet. "The need for central coordination", he said, "would become acute if the country were faced with a sudden crisis involving science and technology . . . the Government would have to deal with a number of awkward questions . . . which did not fit easily into the remit of one research council or ministry . . . the chief scientific adviser would know his way round Whitehall, and he would know how to get the best scientists to work, both inside and outside Whitehall." The post of Chief Scientific Adviser has been vacant since Sir Alan Cottrell returned to academic life more than a year ago. □

Reforms suggested for French Academy

from the staff of La Recherche

A report on the French *Academie des Sciences*, completed in the summer but so far not made public, has made recommendations which have already given rise to some controversy and criticism, particularly from the present academicians.

The report, from a small committee headed by P. Aigrain which was asked earlier this year by President Giscard d'Estaing to study principles and methods of reform, consists of a series of suggestions aimed at giving the Academy a more important role and one similar to that of some other national academies.

The report says that reform of the Academy must begin with lowering the average age of members, which is currently 72. The committee studying ways and means has proposed that, on attaining the age of 70, academicians should be invited to take an "emeritus" position, and retire with a pension. The number of academicians should also be gradually increased, over a period of three years, to 175. The number of "corresponding members" would be raised to about 400, and of foreign "associate members" to about 50. This increase in the number of academicians would pose a serious problem, however, in that, if spread over only three years, it would necessitate 25 to 30 elections per year.

The Aigrain report also recommends the reorganisation of the present inadequate divisions within the Academy. It advocates the creation of two divisions, with mathematics and physical sciences grouped together, and a second group combining chemistry, the life sciences and maybe the social sciences. A fixed percentage of academicians' seats would be reserved for representatives of the applied sciences.

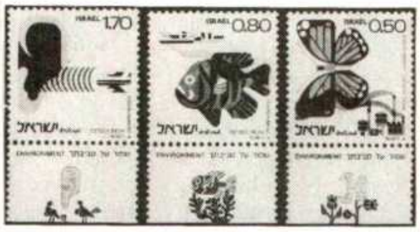
Other less drastic measures are also proposed: an increase in the next budget of the Academy to give it real autonomy; the election of a president every three years, with the option to renew the term of office (the current term is one year); and the creation of posts for foreign researchers invited by the Academy to spend some time in France as "Academy Professors".

Although many of the present members of the Academy think the reforms suggested are too stringent, particularly the proposals to bring in more young blood, and although they oppose the Aigrain report, the Academy's place in French society has not always remained unquestioned. Founded in 1666 on the initiative of Colbert, it played a major part in French science for more than a century. The academicians with their pensions were in some ways the first professional researchers. Instructed by the government to carry out various scientific and technical studies, they did on occasions provide a real scientific advisory service.

The Academy's influence subsequently waned, and governments since 1945 have built new mechanisms for scientific policy which have effectively removed the Academy from decision-making centres and have caused the French scientific community to question its capacity to represent them.

The Minister for Industry and Research and the Secretary for State responsible for the universities must now decide on which measures to adopt. They will doubtless give in on one point at least, that the lowering of the age range could be spread over five years instead of three. However, since the Academy has elected a relatively young man, Professor P. Germain, to the post of Permanent Secretary, to replace L. de Broglie on his resignation, it appears that, even if with rather bad grace, the Academy is ready to accept a large part of the proposed reform. □

Occasionally the animal kingdom seems to decide on its own to fight back against the manifestations of modern civilisation. In northern Israel recently, Syrian woodpeckers actually destroyed kibbutz irrigation systems. Apparently, they find the sound of water rushing through plastic irrigation pipes similar to the sound made by various insects inside trees, and so peck away at the plastic as if it were wood. Because the woodpeckers did not stop after failing to discover any insects, people are wondering whether the birds have developed a taste for plastics. Although distressing for the farmers, the development has at least shown that traditional plastics may be biodegradable after all.



Stamping out pollution

Israel's environmental problems are particularly severe because of a decades-long tendency to disregard ecological considerations in an attempt to achieve rapid development at almost any cost. This now seems to be changing, if the establishment of several bodies to deal with environmental deterioration is anything to go by. A series of stamps (above) has also been issued on the subject. They deal with air pollution (a butterfly engulfed by factory fumes), water pollution (a fish swimming in waste discharged by a ship) and noise pollution (an ear, presumably human, under assault by sound waves from a jet). Tabs below the stamps show the butterfly in the company of flowers rather than fumes, the fish next to an underwater plant rather than a ship, and an ear serenaded by birds rather than jets.

from Nechemia Meyers

Peer-review under review

by Colin Norman, Washington

IN response to a mounting chorus of complaints from a few vociferous members of Congress, and background grumblings from a number of scientists, the two government agencies chiefly responsible for supporting basic scientific research in the United States have launched an important inquiry into the methods used for reviewing grant applications. The National Science Foundation (NSF) and the National Institutes of Health (NIH), which together spend $2,500 million on research each year, have begun three separate studies of their grant-award systems. The results, which should be available next year, will probably provide at least a partial vindication of the agencies' procedures, but some fundamental principles are at stake, and some changes can be anticipated.

At the heart of the inquiry is the so-called peer-review system, which is used in some shape or form by virtually every government agency which supports academic research. Although it has never lacked critics, the system has been vigorously defended by most scientists as the fairest, and probably the best, method for judging the relative merits of competing grant proposals.

NSF handles some 21,000 grant proposals each year, nearly three quarters of which are sent out by mail to several scientists to review. A third of those are also reviewed by a panel of scientists at a meeting. Most of the rest are reviewed by a panel only, frequently with the applicant along to discuss his proposals, while a few are not peer-reviewed at all. NIH grant applications are nearly all reviewed by panels of scientists. In both cases, officials from the agencies ultimately decide which applications will be funded.

Traditional defences of the system have clearly failed to mute the criticisms. In fact, the reverse seems to have happened. Thus, NSF last month sent out 4,500 questionnaires to grant applicants and reviewers of grant proposals to gauge the feelings of the scientific community toward its grant awarding mechanisms, and it has also asked the National Academy of Sciences to undertake an independent investigation of the peer-review process. Meanwhile, NIH has established a top-level internal committee, under the chairmanship of Dr Ruth Kirschstein, director of the National Institute of General Medical Sciences, to conduct a broad evaluation of NIH's grant system.

The two agencies have been pressed into their investigations by an extraordinary series of political attacks on basic research from congress throughout the past year. NSF was thrown on the defensive about a year ago when Senator William Proxmire, who has previously attacked the Pentagon budget, criticised a few NSF-supported research programmes which he considered to be a waste of taxpayers' money.

By publicising trivial or humorous grant titles, and their costs, Proxmire ensured wide press criciticism of NSF, which in turn made people ask why Congress wasn't keeping a closer watch on NSF activities. The House of Representatives attached a provision, later dropped, to an NSF budget bill which would have given Congress a chance to vet every research project which NSF wanted to support, and to veto those which the politicians didn't like. It sent cold shivers through the scientific community.

But that was only the beginning. NSF has more recently found itself in hot water with right-wing congressmen because of its sponsorship of some controversial school science courses. Led by a conservative Republican from Arizona, John B. Conlan, the critics began by attacking the courses and railing against NSF's role in developing and promoting them. But their criticism turned into a broader attack on NSF's peer review system when Conlan was denied access to peer-review reports on one NSF course which he considered particularly offensive. He then turned his attention to the secrecy of the peer-review process which, he asserted, makes it impossible for Congress or the public to hold NSF accountable for its actions.

The matter eventually came to a head last summer, when the House Committee on Science and Technology held two weeks of hearings on NSF's peer-review process, in the course of which Conlan and others presented their thesis that peer-review, as used by NSF, provides too much power to NSF officials, stifles innovative proposals and gives rise to what Conlan described as "an incestuous buddy system". Following the hearings, Conlan and Senator Jesse Helms, a conservative Republican from North Carolina, introduced similar bills into the House and Senate calling for a radical overhaul of the system at NSF which, among other things, would ensure that verbatim copies of peer-review reports, complete with the name of the reviewer, should be made available to the applicant and to Congress.

NSF, in the meantime, has been busy preparing its defence and making some concessions. The National Science Board (NSB), NSF's governing council, has decided that, beginning in January, grant applicants should be able to obtain verbatim copies of the reports of reviewers of their grant proposals. The NSB also decided, however, that, for the moment, the names of the reviewers should remain confidential, a policy which NSF's director, Dr H. Guyford Stever, has defended on the grounds that many scientists would be unwilling to review grant proposals if they are not guaranteed anonymity, and that open reviews would be less candid. It is partly to judge the accuracy of such assertions that NSF is soliciting the opinions of scientists concerning its review procedures.

The NSB, in conjunction with the House Science and Technology Committee, drew up the questionnaires and mailed them out to equal numbers of successful and unsuccessful grant applicants and reviewers. The results will be analysed early next year by a Congressional agency, the General Accounting Office, and they will provide the first comprehensive indication of the attitudes of scientists towards the peer review process.

In the meantime, the National Academy of Sciences' Committee on Science and Public Policy (COSPUP) has entered into a contract with NSF to conduct an independent investigation of the peer-review process. Its first report, describing the system and pinpointing its strengths and weaknesses, should be completed next summer, and a later report will deal with the manner in which the results of NSF-supported research are used.

As for NIH, it has been spared most of the recent congressional criticism of the peer-review process, but there have nevertheless been repeated grumbling about its granting mechanisms from a variety of sources. Consequently, top NIH officials decided to establish the internal review committee. Its mandate is a broad one. It has been asked to look into the philosophy behind NIH's peer-review process and to recommend necessary modifications, to examine alternatives to the system, to investigate the effects of recent legislation calling for more openness in governmental decision-making on peer-review and to look into the possibility of establishing a formal appeals process for disgruntled grant applicants.

The Committee is concerned only with the procedures used to evaluate grant proposals, not with NIH's growing commitment to contract research. It is hoping to hold three sets of public hearings in February to solicit the ideas of NIH's clients in the universities, and its final report is due by June 30 next year.

Whatever the various investigations of the peer-review process finally conclude, some matters dear to the hearts and professional well-being of the scientific community are at stake. □

news and views

Chirality and the origin of life

from Tilo L. V. Ulbricht

ONE of the unsolved problems (there are many) concerning the origin of life is the choice by living organisms of one set of optical isomers: as is well known, only L-amino acids are found in proteins, whereas laboratory syntheses from optically inactive starting materials yield 1/1 (racemic) mixtures of L- and D- isomers. One can understand how the optical purity of living organisms is maintained, once it exists, because enzymes, composed exclusively of L-amino acids, catalyse the formation of only the desired isomer. Moreover, many organisms even have a special enzyme, D-amino acid oxidase, to remove the unwanted isomer. But how did it all begin?

Could there have been some asymmetric factor in the environment which influenced the choice? It has been suggested that life began in the vicinity of optically active quartz crystals, but then the choice of L- or D- was merely a matter of chance. Another suggestion is that circularly polarised light might have been the agent. Many years ago, Kuhn showed that circularly polarised light could induce optical activity by asymmetric decomposition, and much more recently true asymmetric synthesis has been accomplished using circularly polarised light. But consideration of the interaction of the Sun's radiation with its own or with the Earth's magnetic field, and the fact of the frequent reversals of the Earth's magnetic field, indicates that there was no predominant handedness in the circular polarisation of incident sunlight over any significant time spans.

A further possibility is an asymmetry in the environment from natural radioactivity. There must have been quite a few scientists who were struck by the analogy between the asymmetry at the level of elementary particles (left-handed electrons and right-handed positrons) and asymmetry at the molecular level, when the discovery was announced in January 1957 that parity was not conserved in β decay. But the existence of the analogy does not mean that any causal relationship exists. Vester and I examined this possibility, suggested some mechanisms and early in 1957 carried out experiments to try and induce optical activity in chemical systems under the influence of polarised (left-handed) β rays. (For a review of the earlier literature, see Ulbricht, *Origins of Life*, **6**, 303; 1975.) One of the mechanisms proposed was the following: longitudinally polarised β rays→ circularly polarised bremsstrahlung → optically active molecules.

As had been predicted by Lee and Yang, the ultraviolet radiation produced when β rays slow down in matter (bremsstrahlung) is circularly polarised if the β rays are polarised. Thus, at least in principle, a route from polarised electrons to optical activity exists. The early experiments, however, were negative or inconclusive, although in retrospect one result was interesting. This was an experiment in which D, L-alanine was mixed with Ag powder and irradiated in a neutron pile, conditions in which the radioactive isotopes ^{108}Ag and ^{110}Ag are formed. Ninety-six per cent of the alanine was destroyed, and the soluble residue had a very small rotation which was not considered significant.

Because of these inconclusive results and the, as it then seemed, rather far-fetched nature of the hypothesis, there was little interest in the idea for some years. Two quite independent factors stimulated new activity. The first was the realisation by physicists that parity violation occurred not only in weak interactions like β decay (which have little to do with chemistry) but that there is also a small parity-violating component in all electromagnetic interactions—which are, of course, at the very basis of chemistry. This was strengthened by the discovery of neutral currents, suggesting that weak and electromagnetic interactions may have a common foundation. One consequence of this is the realisation that optical isomers do not have exactly equal energy contents. Some experiments designed to amplify this small difference have yielded positive results (see Ulbricht, *op. cit.*). The second factor was a paper by Garay (*Nature*, **219**, 338; 1968) reporting that solutions of D- and L-tyrosine decompose at different rates in the presence of β rays from ^{90}Sr-Y. This was the first evidence that polarised electrons could distinguish between L- and D-isomers.

Vester and I had thought it unlikely that a direct interaction between high-energy electrons and molecules could produce optical activity, and similarly, Ulrich and Walker (this issue of Nature, page 418) argue that stereospecific discrimination is unlikely in high energy processes. They think, therefore, that if asymmetry from β rays is communicated, it must be by way of the secondary electrons created during their energy degradation. Accordingly, they have searched for evidence of optical activity in solvated electrons, supposing that an electron confined to a helical solvent may show a different probability of reaction with D and L isomers. No evidence of such a difference was found, or of optical activity of solvated electrons in an optically active solvent. These negative results would seem to rule out certain mechanisms involving secondary electrons, though it should be noted that differences in rate constants of less than 10% could not have been detected.

However, several recent investigations have produced positive results. Kovacs and Garay (*Nature*, **254**, 538; 1975) did a large series of crystallisations of D,L-sodium ammonium tartrate in the presence and absence of ^{32}P-phosphate, measuring the optical activity of the crystals. Whereas a virtually symmetrical distribution of optical activity about zero was obtained in the control series, in the presence of ^{32}P the distribution was markedly asymmetric, the L-salt crystallising preferentially.

In view of the doubts about some of the previous experiments in this field, the results of Bonner *et al.* (page 419, this issue) will attract particular attention. Using a special apparatus which could produce either left-handed ("natural") or right-handed ("unnatural") electrons of 10–23% polarisation, solid D,L-leucine was irradiated to 50–75% decomposition. Determination of the enantiomeric composition of the undecomposed leucine residue showed that the left-handed electrons (those having the same anti-parallel spin as natural β rays) brought about the degradation of D-leucine more than of L-leucine; and what is most striking, right-handed electrons decomposed L-leucine more than the D-isomer. Thus, the "natural" electrons produced an asymmetric degradation favouring the "natural" amino acid, and *vice versa*.

From the results reported in the last few months it seems reasonable to conclude that β rays can indeed induce optical activity. What remains totally obscure is the mechanism; for example, in the experiments by Bonner *et al.*, was the effect a direct one of the polarised electrons themselves, or was it due to bremsstrahlung? ☐

Transposable resistance genes

from J. R. Saunders

It is increasingly evident that certain bacterial resistance genes are capable of migration between DNA molecules. This ability makes it easier to understand the rapid evolution of antibiotic-resistance (R) plasmids possessing varied combinations of linked resistance genes and may indicate a more general evolutionary process in bacteria and other organisms.

The curious ability of a gene specifying ampicillin-resistance to be translocated to different replicons in bacteria has been known for some time (see, for example, Anderson, *CIBA Symposium on Bacterial Episomes and Plasmids*, 102, 1969; Richmond and Sykes, *Genet. Res.*, **20**, 231; 1972). Hedges and Jacob (*Molec. gen. Genet.*, **132**, 31; 1974) demonstrated that the ampicillin-resistance gene present on the plasmid RP4 can be acquired by a variety of other plasmids co-existing in the same strain of *Escherichia coli*. Plasmids acquiring this gene showed concomitant increases in molecular weight, and were in turn able to translocate the gene, which they termed Transposon A (TnA), to other plasmids. Recently a number of other resistance genes capable of translocation between chromosomal, phage and plasmid DNA have been identified. These include, for example, kanamycin-resistance (Berg *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3628; 1975), tetracycline-resistance (Kleckner *et al.*, *J. molec. Biol.*, **97**, 561; 1975) and linked resistance to streptomycin and trimethoprim (Hedges *et al.*, *Mol. gen. Genet.*, **140**, 289; 1975). This suggests that discrete translocatable elements (transposons) may constitute relatively common genetic units in bacteria.

Transposons are apparently able to insert into and excise from DNA molecules in the absence of a functional bacterial recombination (*rec A*) system. This suggests that such elements use alternative, possibly self-specified enzymes for recombination. Despite this partial independence, transposons have not been shown to exist autonomously and need to reside in a functional replicon.

A clue to the mechanism of insertion and excision comes from electron microscope studies of DNA heteroduplexes formed between phage or plasmid DNA with and without inserted transposons. These indicate that most transposons are composed of a resistance gene (or genes) flanked on either side by inverted complementary DNA sequences (inverted repetitions) of between 100 and 1,500 nucleotide pairs. Some, but not all such repetitions are homologous with known insertion sequences (IS). For example, the inverted repetitious sequences bounding the transposable tetracycline-resistance gene are homologous with IS3. (Ptashne and Cohen, *J. Bact.*, **122**, 776; 1975). Insertion sequences are present at various sites in both plasmids and the chromosome of *E. coli* (Hu *et al.*, *J. Bact.*, **122**, 764; 1975) and have been implicated in a number of *rec A*-independent and illegitimate recombination events.

Insertions of transposons are mutagenic when occurring in structural genes and polar when occurring within operons. This is also a general property of insertion sequences (Starlinger and Saedler, *Biochimie*, **54**, 177; 1972). As shown by Klechner *et al.* insertion itself is a relatively precise process which does not damage the informational capacity of host DNA. On the other hand, excision often results in permanent loss of host genetic information. Heffron and colleagues (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 3623; 1975) show that TnA is able to insert at a minimum of twelve distinct sites on the genome of the plasmid RSF1010, suggesting recognition by the transposon of a specific but fairly common nucleotide sequence at or near to the point of integration. Insertion/excision must be by a mechanism which conserves the inverted repetitions since transposons can be repeatedly translocated whilst retaining such sequences. This could occur if excision involves recombination between the two flanking inverted repetitions such that one of the 'arms' of the repeat is retained by the genome and the other by the looped-out resistance gene. The latter could then circularise and migrate either to another DNA molecule or to a different site on the same molecule. Provided that a homologous repeat sequence is present on the recipient molecule insertion is then possible by recombination between the two repeats. It should be pointed out however, that circularised transposons have not yet been detected in the process of migration, which suggests that such a stage, if present, is short-lived.

The significance of transposition is that it allows rearrangement and recombination of certain genes from heterologous genetic backgrounds. The phenomenon also provides a neat mechanism for the accretion of resistance genes by sex factors to form infectious multiple resistance plasmids. It is known that relatively few types of resistance gene are common to a wide variety of R plasmids, found in different bacterial species, and believed on both genetic and physical grounds to be of disparate evolutionary origin. For example, the β-lactamase (penicillinase) gene of TnA, and base sequences homologous with it, are widely distributed on an assortment of plasmids (Heffron *et al.*, *J. Bact.*, **122**, 250; 1975). This suggests that all have received the resistance gene from a common source. Transposons presumably evolved initially in their ancestral host by association of resistance genes with inverted repetitious sequences. Such elements could then be attached to a variety of replicons, including genetic vectors and hence find their way to other bacterial strains where they could be retransposed to resident DNA molecules. This process is of enormous evolutionary advantage because it obviates the need for the gross genetic homology normally required for recombination and allows reassortment of genes on different replicons. Therefore tandem insertion of numbers of resistance genes into a plasmid of unrelated nucleotide sequence would be possible, provided that the relatively short relevant inverted repetitions were present.

Transposons may prove powerful tools for genetic research since attachment of a resistance gene to a DNA molecule provides it with a readily discernible genetic and physical tag. It will be of great interest to discover the precise mechanism of their insertion and excision. Ways of doing this would include sequencing of the inverted repeats, particularly at their functions with non-repetitious DNA, and searching for a specific enzymatic machinary for mediating insertion and excision. It seems likely that the latter will involve enzymes with the specificity of restriction nucleases for recognising insertion sites. In view of the frequency of inverted repetitious DNA in both prokaryotes and eukaryotes, transposons may represent a more general class of genetic elements contributing greatly to evolutionary flexibility. ☐

Classical interferometry and quantum statistics

from J. Byrne

In the half-century since the advent of de Broglie's theory of matter waves many striking and beautiful demonstrations have witnessed to the truth of this hypothesis. Under various shapes and guises the classic experiments of Young and Fresnel have been reproduced with particle beams, the motivation, at least in part, being the desire to determine the limits within which these observations may be encompassed by the framework of a classical wave field. Experiments which differentiate between classical and quantum wave phenomena are rare exceptions, and the interest they excite is correspondingly greater. A recent variant (Werner *et al.*, *Phys. Rev. Lett.*, **35**, 1053; 1975) of Young's double slit experiment, which establishes in a direct and unambiguous way the fermion character of neutrons, falls squarely in the rare category—a new departure in a field with a long and distinguished history of innovation.

Angular momentum algebra, the language of rotations, is not a topic which normally rates a mention in treatises on interferometry, but in the present context it provides a useful starting point. The notion of a vector is familiar in classical physics as an entity, which, having magnitude and direction, is characterised by three real numbers. In quantum systems of spin S the number of components is $2S+1$ and by this criterion vector fields have unit spin. However S is also permitted to take half-integral values; this brings in spinor fields of which two component systems are the most prominent. The spin-statistics relation, which associates integral spin with bosons and half-integral spin with fermions, rests on the firmest of theoretical and experimental grounds.

For the operation of rotation to have a meaning for spinors the components are necessarily complex; the additional degree of freedom implies that spinors may be assigned a phase in addition to their magnitude and direction. An unavoidable consequence is that spinors undergo a sign change under rotation through an angle of 2π about any axis, the property which is of current experimental interest.

Uncharged and having a small magnetic moment, the neutron represents an ideal fermion system in which to detect the phase difference between coherently superposed spinor fields which have been subjected to a relative rotation. (Bernstein, *Phys. Rev. Lett.*, **18**, 1102; 1967; Aharonov and Susskind, *Phys. Rev.*, **158**, 1237; 1967). In a magnetic field the two spin states have energies $\pm\frac{1}{2}\hbar\omega_L$, where ω_L is the angular frequency of Larmor precession. Because the force field is conservative the magnetic energy is compensated by a change in kinetic energy and the free spinor

$$\mathbf{U}_o=\begin{pmatrix}a_+\\a_-\end{pmatrix}\exp\,(ip_o x/\hbar)$$

is transformed in a magnetic field to

$$\mathbf{U}_H=\begin{pmatrix}a_+\exp(i\surd(p_o{}^2-m\hbar\omega_L)\,.\,x)\\a_-\exp(i\surd(p_o{}^2+m\hbar\omega_L)\,.\,x)\end{pmatrix}$$

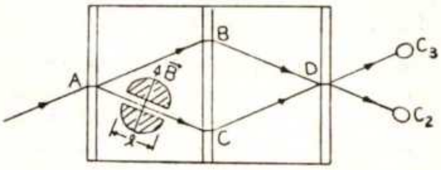
$$\simeq\begin{pmatrix}\exp(-i\omega_L x/2p_o) & 0\\0 & \exp(-i\omega_L x/2p_o)\end{pmatrix}\mathbf{U}_o$$

to first order in $\hbar\omega_L/(p_o{}^2/2m)$. The transformation matrix represents a rotation of the spinor through an angle $\varphi=m\omega_L x/p_o$.

The coherent superposition of $\mathbf{U}_H$ and $\mathbf{U}_o$ results in the spinor $\mathbf{U}_C=\mathbf{U}_H+\mathbf{U}_o$ for which the intensity is

$$I=|\mathrm{U}_{C+}|^2+|\mathrm{U}_{C-}|^2=4(1+\cos^2(\varphi/2))$$

The minimum at $\varphi=2\pi$, which corresponds to a shift of half a fringe in the interference pattern as $\mathbf{U}_H$ rotates through an angle of 2π, is a direct consequence of the two-valued nature of spinors. Such an effect would not be observed for vector particles such as the metastable orthohelium atom.



A schematic diagram of the neutron interferometer. On the path AC the neutrons are in a magnetic field B (0 to 500 G) for a distance l (2 cm).

The very simplicity of these ideas tends to mask the formidable technical problem presented by a laboratory investigation in which the combination of spatial coherence in the two beams with total spatial separation is of the essence. The system adopted by the experimenters, and illustrated in the figure, bears some resemblance to an optical interferometer of Mach-Zehnder type. A symetrical Laue reflection at B gives two coherent beams with an angular separation of twice the Bragg angle; following further reflections in the mirror BC the beams are recombined in the analyser AD. All elements of the interferometer are formed by cutting grooves in a single dislocation-free crystal of silicon. One of the component beams propagates normal to a magnetic field and the resultant interference pattern is observed by recording the counting rates at C_2 and C_3. The crucial advantage which the interferometer enjoys over its optical counterpart stems from the use of diffraction for purposes of amplitude division which, for small Bragg angles, results in an achromatic system of fringes (Bonse and Hart, *Appl. Phys. Lett.*, **6**, 155; 1965). In these circumstances the coherence length is effectively infinite and the Laue technique may be employed in a broad band of neutron energies.

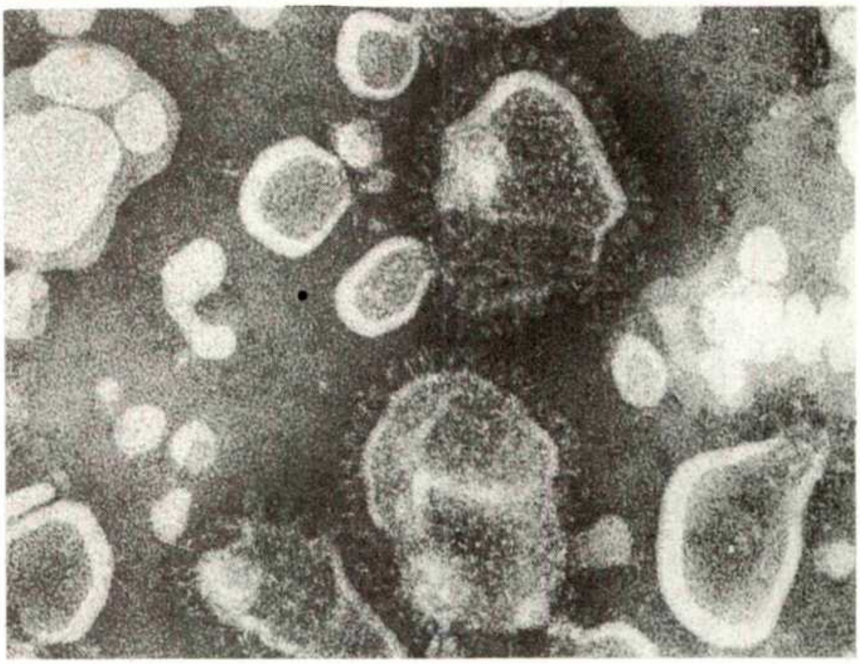
To conclude, two brief observations may be made. First, the rotation of the spinor $\mathbf{U}_H$ may be established experimentally by measuring the polarisation of those neutrons which traverse the magnetic field. On the other hand the interference effect is observed with the spinor $\mathbf{U}_C$ and for these neutrons the polarisation is turned through an angle of π not of 2π. There is therefore no question of making simultaneous observations of the spin rotation and of the phase difference. The second comment refers to the unitary property of the rotation matrix for spin $\frac{1}{2}$ fermions which implies that an equivalent interference effect should be observable for any two-state system, fermion or boson, which is taken through a full cycle of unitary transformations. It must be emphasised however that only in the case of fermions can such a cycle of change be understood as a spatial rotation. □

Canary mantle plume unlikely

from Peter J. Smith

So much attention has been given to the Hawaiian–Emperor island chain in connection wtih the mantle plume–hot spot hypothesis that there is a tendency to forget the other areas in which similar ideas may be put to the test. One example is the group of seven Canary Islands. Not that the Canaries have been overlooked entirely. Morgan (*Nature*, **230**, 42; 1971), for example, located a mantle plume beneath the islands; Burke and Wilson (*Nature*, **239**, 387; 1972) suggested that the islands were formed over a plume active for the past 22 million years; Wilson (*Tectonophys.*, **19**, 149; 1973) classified the Canaries among "young hot spots on ocean floors that may be stationary"; and Schmincke (*Geol. Soc. Amer. Bull.*, **84**, 634; 1972) concluded that although there were insufficient data to confirm a hot spot origin, there was nevertheless some support for it.

But in spite of this interest, Anguita and Hernan (*Earth planet. Sci. Lett.*, **27**, 11; 1975) claim that, with the possible exception of Schmincke, no one has really examined the nature of the Canary island chain in any great detail. Certainly it has not been subjected to anything like as much study as the Hawaiian islands. The unfortunate result is that the Canary group "has been incorporated to the geo-



VIROSOMES—Surface subunits of the influenza virus attached to unilamellar liposomes (Almeida *et al.*, *Lancet*, **ii**, 899; 1975). Anti-influenza serum aggregates the virosomes, indicating that most of the original binding sites are still available. The authors point out that virosomes may be of use in solving some of the problems associated with vaccination against influenza. The whole inactivated influenza virus, although a good immunogen, is pyrogenic, but isolated subunits, although non-pyrogenic, are not good immunogens. Preliminary experiments indicate that the liposomes may provide the required adjuvant without pyrogenicity (×275,000).

logical literature [as an example of plume activity] after little or no discussion of the chronological, bathymetric, petrological and tectonic data". Anguita and Hernan have therefore examined such information, where it exists—and conclude that the Canaries are probably not the result of plume activity at all.

Superficially, the Canary Islands qualify as a potential plume effect. The trend of the seven islands is in the direction of the local seafloor spreading and the radiometric ages of the oldest measured subaerial volcanics on each island decrease generally from one end of the chain to the other. But under closer examination the oldest-age data tell a less convincing story. First, they are not necessarily complete. The deepest levels of the exposed outcrops may not represent the earliest subaerial activity; and in one island (La Palma) the oldest dated samples do not even come from the deepest subaerial section. Second, although seafloor spreading rates quoted for the eastern Atlantic generally lie between 1 and 2 cm yr^{-1}, the apparent plate velocities calculated from the different pairs of islands in the Canaries (assuming any implied plume to be stationary) range from 0.6 to 26.7 cm yr^{-1}.

Another difficulty is that the full range of available age data (that is, not just those from the oldest subaerial flows) indicate a hiatus of 3–8 Myr in the subaerial volcanic activity on each island. In short, the subaerial volcanism is split into two distinct episodes: the first, which follows the submarine phase, and the second, which follows the hiatus.

An important feature of the three phases (one submarine, two subaerial), however, is that a trend towards greater alkalinity with time is observed within each. In the case of Hawaii, a similar (single) trend has been interpreted in terms of progressive movement away from a melting zone and thus in terms of a mantle plume beneath a moving plate. But the existence of three separate geochemical cycles appears to make nonsense of such a concept as applied to the Canary volcanics, except in the highly unlikely event of oscillating plate motion.

So if the Canaries are not derived from a plume, what is their origin? Anguita and Hernan clearly favour a propagating fracture of the sort proposed by McDougall (*Nature*, **231**, 141; 1971) and caused by the tensions implicit in the "membrane tectonics" of Turcotte and Oxburgh (*Nature*, **244**, 337; 1973). The basic idea is that a developing lithospheric fracture, tapping the asthenosphere, will allow volcanism to spread along it until the existing stresses are released. In the case of the Canary Islands, however, the fracturing process would have to be repeated several times and fracturing would propagate with a non-uniform velocity.

Such a model seems to be consistent with tectonic trends (in both space and time) in West Africa. The directional trend of the western and central Canary Islands aligns more or less with the continent South Atlas Fault, a circumstance which leads Anguita and Hernan to suggest that the young oceanic fracture reflects the influence of the older system. Moreover, the western High Atlas structures of Africa were generated largely in three important orogenic pulses (in the Upper Eocene, the Upper Oligocene and the Miocene, respectively) which correlate well in time with the three distinct phases of activity in the Canaries (with each compressive pulse preceding a magmatic phase). Anguita and Hernan thus propose that it was the tensional period between each compressive pulse which allowed the build-up of stress and hence the failure of the lithosphere and the consequent upwelling of magma. Similar arguments may be adduced for the western Canary Islands, except that the directional trend involved here is slightly different.

Ultimately, however, the most interesting, if not crucial, evidence against the plume hypothesis may prove to be the least direct. There has recently been much talk about possible stationary states of Africa. A proven stationary Africa and a simultaneously moving volcanic centre in the Canaries would presumably rule out mantle plumes, or at least fixed plumes, for good.

Protein structures without heavy atoms

from David Blow

UNTIL recently all detailed knowledge of protein structure came from crystallographic studies using the isomorphous replacement method. This requires a laborious screening of crystals soaked in a variety of heavy metal solutions, to see whether any provides a suitable derivative. Techniques for direct structure determination by analogy with another protein of known structure were worked out in the sixties, but had not been used successfully, and their usefulness was doubted. These doubts are finally destroyed by the redetermination of the structure of trypsin by Bode, Fehlhammer and Schwager (*J. molec. Biol.*, **98**, 683, 693; 1975).

Previous attempts along these lines had produced little new information because of the limited resolution. Seal myoglobin and lamprey haemoglobin were shown to be close structural relatives of sperm whale myoglobin. Slightly more information was gained about carboxypeptidase B, which was shown in a study at 5.5 Å resolution to lack electron density at the N-terminus by comparison with carboxypeptidase A, which has four extra N-terminal amino acids (Schmid *et al.*, *J. molec. Biol.*, **84**, 97; 1974).

Although these methods served to confirm that one structure was like another, they were unable to provide detailed information about the new structure. Bode *et al.* show such information can be gained if one works at sufficiently high resolution.

The Munich group had already worked out one structure involving the trypsin molecule. This was the complex of pancreatic trypsin inhibitor with trypsin (Huber *et al.*, *J. molec. Biol.*, **89**, 73; 1974). They used isomorphous replacement to give an electron density map, and then used a computer method to orient the inhibitor structure, which they had worked out separately, to give the best fit to the map. For the trypsin part of the complex, a previously determined trypsin structure did not fit well, but the coordinates for chymotrypsin, edited to account for known chemical differences, could be rotated to a correct orientation and refined. During refinement, the phase angles obtained from isomorphous replacement were abandoned in favour of phase angles calculated from the partly refined structure.

This is the key point of the whole operation. At lower resolution, the insertion of calculated phase angles would firmly fix the structure at that assumed in the phase calculation. This work was done at 2.3 Å resolution, where there is sufficient detail in the electron density map to require every atom to be in the right place.

In the latest work, the trypsin structure obtained from the complex was used as a starting point. Instead of isomorphous replacement, a search was made for the best orientation of the trypsin molecule to fit the diffraction data. This orientation was used to provide calculated phases, and the procedure continues as before, this time at 1.8 Å resolution.

The results show a remarkable consistency. The two trypsin structures have a mean difference of 0.25 Å (though due to error in the reorientation of the trypsin molecule, the starting orientation averaged 0.44 Å away). Main chain atoms common to chymotrypsin and trypsin differ on average by 0.75 Å, and most side chains and internally bound water molecules are in similar conformations. Considering the different conditions, and the fact that these comparisons must include experimental error, they indicate that the polypeptide chain of these enzymes has evolved considerable rigidity.

Working at a lower resolution of 3.5 Å, Ward *et al.* (*J. molec. Biol*, **98**, 161; 1975) have determined the structure of new crystal forms of human deoxyhaemoglobin A and S, using similar techniques. At this resolution no refinement was possible, and the authors are rightly cautious in interpreting apparent differences between their structures (crystallised in polyethylene glycol solutions) and the normal form. The particular interest of these structures is that they may provide a clue to the mechanism by which sickle cell haemoglobin aggregates into fibres.

These techniques mean that a new

Brightly shines the X-ray quasar

from John Gribbin

A NEW candidate for the title "intrinsically the strongest X-ray source known" has been put forward by Schreier *et al.* on the basis of observations made by the Astronomical Netherlands Satellite (ANS) and a timely revision of an old Uhuru error box (*Astrophys. J. Lett.*, **201**, L137; 1975). If the identification is correct, and if the QSO NAB0137–01 which is the subject of the identification is at the cosmological distance implied by its redshift of 0.33, then the source radiates about 2×10^{46} erg s^{-1} at X-ray frequencies, four times as much as 3C273 and 50 times as much as its own optical luminosity.

But there must remain some doubt about the identification of the Uhuru source 3U0138–01 with this QSO, even though the evidence is suggestive. A tentative identification had already been made on the basis of the original Uhuru data, and this now looks better since the Uhuru error box first published turns out to have been too short in one direction; the stretch in the box implied by subsequent revisions brings one edge almost out to include the QSO. The strip of sky scanned by ANS definitely includes the QSO, but the triangular error box resulting from a combination of Uhuru and ANS observations still does not quite include this object at the 90% level.

The QSO was originally found by Bahcall *et al.* (*Astrophys. J. Lett.*, **199**, L9; 1975) during an optical search of the sky near 10 Uhuru high latitude sources. They estimate that 0.7 QSOs would be found among the 10 boxes by chance, and the location of NAB0137–01 just outside the 90% error box for 3U0138–01 seems to fit this estimate rather well. Schreier *et al.*, however, point out that with the ANS data this particular error box is now much smaller, and say that the statistical chance of finding Bahcall *et al.*'s 0.7 of a QSO in this particular box out of the 10 is much smaller. The combined ANS/Uhuru box covers 0.02 square degrees, while the other nine Uhuru boxes of this search range from 0.14 to 0.52 square degrees in size. The probability that a QSO would be found by chance in a box of 0.02 square degrees is only 0.005; but the statistical argument remains unsatisfactory, as is usually the case for samples of one, and further identifications of high latitude X-ray sources with QSOs are needed before this can be taken at face value.

Even so, the possibility that NAB0137–01 has such a large X-ray to optical luminosity ratio is interesting in view of the lack of other identifications. It suggests that the optical objects associated with such X-ray sources may be very faint, and highlights the need for searches down to limiting magnitudes of at least 18—as well, of course, as emphasising the need for better X-ray positions.

THERE has just been placed in Westminster Abbey a marble scroll bearing an appropriate inscription to the memory of Jeremiah Horrocks The movement for such a memorial was commenced some time ago, and is referred to in NATURE, vol. x. p. 190, and xi. p. 31. The inscription is as follows:—

In Memory of
JEREMIAH HORROCKS,
Curate of Hoole, in Lancashire,
Who died on the 3d of January, 1641,
in or near his 22d year,
Having in so short a life
Detected the long inequality in the mean
motion of Jupiter and Saturn;
Discovered the orbit of the Moon
to be an ellipse,
Determined the motion of the lunar apse,
Suggested the physical cause of its revolution;
And predicted from his own observations
the Transit of Venus,
Which was seen by himself and his friend
William Crabtree
On Sunday the 24th of November (O S) 1639
This Tablet, facing the Monument of Newton,
Was raised after the lapse of more than
two centuries, December 9, 1874

from *Nature*, **13**, 112, Dec. 9, 1875.

avalanche of protein crystal structures is on its way, hastened by the automatic techniques now available for data collection. Travellers to the East Coast Protein Crystallography meeting in November returned dazed by the overwhelming tide of information, including one structure solved at 3.5 Å resolution, start to finish, since April. But there are many fewer structures which have been determined accurately and pondered deeply. It looks as if these direct methods are only going to give new structural information if they are taken to resolutions beyond 2.5 Å. Even with automated equipment, that comes fairly slowly, and carries with it a hefty computing bill

Health physics instruments, their design and calibration

from R. W. Clarke

A meeting, sponsored by the British Radiological Protection Association and presented by the Society for Radiological Protection was held on October 22 and 23 at the Berkeley Nuclear Laboratories, CEGB.

THERE is seldom a meeting involving any aspect of radiological protection where the basic units of dose measurement are not once again debated, with new systems proposed and old ones denigrated. It is not surprising therefore that this meeting opened with a valiant attempt by H. J. Dunster (National Radiological Protection Board, Harwell) to rationalise current practice by the examination of the objectives of such measurements He declared that dose equivalent index (defined as the maximum radiation dose, corrected for quality and other factors, within a 30 cm diameter sphere consisting of material equivalent to soft tissue with a density of 1 g cm^{-3}) was the only environmental quantity for general application. It should be measured at depths in this sphere such that the superficial tissue was 7 mg cm^{-2} and 1 g cm^{2-} (that is, 0.007 cm and 1 cm). At such depths, corresponding to the doses to skin and internal organs respectively, the result would include the dose to the eyes at 300 mg cm^{-2}. In uni-directional fields, instruments could be made to measure this index accurately or, more importantly for regulatory purposes, reproducibly. It is clear that in multi-directional fields the answers could be commonly a factor of two too high or more but this should be acceptable. More precise measurement of the radiation requires a detailed research examination of fluence and energy quality only justified for post-accident enquiries and similar cases.

Despite the development of new definitions of radiation dose, manufacturers seem to persist obstinately in producing instruments that read in the old exposure units (e.s.u. generated in air) measured in röentgens even though protection regulations are couched in terms of absorbed dose (erg per g of air) measured in rads or rems. D. White (NRPB) while appealing for the cessation of exposure meters, recommended the measurement of absorbed dose in air beneath layers of 300 mg cm^{-2} and 700 or 800 mg cm^{-2}. To those who search for ever more constant energy response in radiation detectors, he demonstrated that if the errors of individual variables, such as angular response, linearity and scales are compounded then a factor of two is arrived at for the accuracy to 95% confidence limits. There seemed little point in concentrating on the improvement of energy response which is only one of many contributing sources of error.

To the simple participant at this meeting, it seems we find ourselves still debating new units and methods of measurement while the actual users and even the national standards remain addicted to the exposure units originally defined half-a-century ago although there is common acceptance that absorbed dose in air will eventually be registered on every meter. These uncertainties are not shared by the instrument designers. While others argue dose definitions, the electronics engineer is eagerly exploiting the contemporary revolution in integrated circuits Amplifiers, power units, discriminators and digitisers are all less than match-box size. Complete dosemeters can be less than pocket-book size. If only the radiation detectors could similarly shrink.

F H. Wells (UK Atomic Energy Authority, Harwell) was enthusiastic on the subject of the new generation of digitised instruments where 1-5 μrad response is converted to a pulse that can be transmitted over long cables with small power requirements. Portable instruments with digital displays are now available for those health physicists modern enough to abandon meters and in the near future dosemeters with micro-processor devices could do all the calculations one might desire. In theory at least, the electronics can produce readings from a dosemeter in any form or units required but a salutary reminder of production costs and quantities to cover development costs was delivered by a manufacturer (S. Donaldson, Eberline).

An example of the high costs of meeting the requirements of the protection regulations for instrument calibration was provided by the electricity generating industry (I. M. G. Thompson, CEGB) which has funded a neutron-producing accelerator, X-ray machines and a multitude of standard isotope sources purely for this purpose. Yet despite this expenditure, there is at present only a tenuous path connecting health physics dosimetry to the national standards of exposure or dose. Reductions of several orders of magnitude are involved in the most common gamma dose measurements. Strenuous efforts are being made by the custodians of the national standards to produce the balloon ion chamber which has been designed to cover the health physics range of measurements (B. Owen, National Physical Laboratory, Teddington). The British Calibration Service is being used to organise and approve a chain of secondary calibration laboratories to transfer the national dose standards to the instrument user. Criteria are soon to be published for the management, equipment and methods for calibration laboratories but quotes from these criteria presented a daunting glimpse of qualified personnel and expensive equipment which few industries could support. It is clear that there will be only a few secondary laboratories.

J. H. Griffiths (UKAEA), speaking for the user, injected a shock of realism by a simple demand for reliability. There was not an anxiety over dose units nor even accuracy but a concern over the prevalence of failures and false alarms. More than one or two false alarms each year can lead people to ignore installed instruments altogether.

Thus the conflict in health physics dosimetry was complete with the purists defining yet new concepts for the measured units while the practical men were still trying to establish such requirements as calibration standards and reliability based on units of the last decade. Even this well-attended meeting could not resolve these problems but most people could at least see the confusion more clearly. □

Erratum

In the article "Histocompatibility antigens and immunoglobulins?" (*News and Views*, **258**, 193, 1975), a printing error in the penultimate paragraph resulted in an omission. The first sentence of this paragraph should read "If the antigen-detergent complex does not contain a four-chain structure then there is the difficulty of explaining the reason for the appearance of *dimers of* the 45,000 chain in SDS polyacrylamide gel electrophoresis."

review article

The place of the australopithecines in human evolution: grounds for doubt?

C. E. Oxnard*

*Although most studies emphasise the similarity of the australopithecines to modern man, and suggest, therefore, that these creatures were bipedal tool-makers at least one form of which (*Australopithecus africanus—"Homo habilis", "Homo africanus"*) was almost directly ancestral to man, a series of multivariate statistical studies of various postcranial fragments suggests other conclusions. Their locomotion may not have been like that of modern man, and may, though including a form or forms of bipedality, have been different enough to allow marked abilities for climbing. Bipedality may have arisen more than once, the Australopithecinae displaying one or more experiments in bipedality that failed. The genus* Homo *may, in fact, be so ancient as to parallel entirely the genus* Australopithecus *thus denying the latter a direct place in the human lineage.*

An important part of today's conventional wisdom about human evolution is based on studies of teeth, jaws and skull fragments of australopithecine fossils. That these fossils are undoubtedly closely related to the human lineage is now engrained into textbooks[1-3]. So close is this relationship judged to be[4,5] that some of the fragments have been placed in the genus *Homo* itself (for example, species "*habilis*", "*africanus*") while others are accepted as parallel but nevertheless very close evolutionary relatives. Some investigators[6] have long disagreed with these viewpoints on the basis of biometric studies comparing living forms and fossil fragments; more recently other workers[7] have also begun to question accepted views because of new finds in Africa.

As postcranial bones referable to australopithecines are described it is perhaps inevitable that, believing relationships to man to be rather close, those particular features in which the postcranial bones resemble man are most emphasised. Thus those parts of the Sterkfontein pelvis which are similar to those of man (the shape of the iliac blade for instance) are a major factor in the claim that these creatures have an essentially human pelvis with a totally[8] (or somewhat less than totally, depending on the worker[9]) human pattern of bipedal walking. Individual ankle bones (the talus) from Olduvai and Kromdraai have been assessed as indicative of established bipedal walking[10] even though they differ from man more than the African apes do[11]. A series of associated foot bones from Olduvai has been reconstructed into a form closely resembling the human foot today[12] although a similarly incomplete foot of a chimpanzee may also be reconstructed in such a manner. Hand bones, also from Olduvai, possess a total of seven features in which they resemble various apes, compared with only three in which they resemble man, but have nonetheless been evaluated as capable of tool-making in a human fashion[13]; this last evidence is now even weaker because of the relatively newly discovered abilities of various apes for using and making tools[14]. A fragment of scapula described many years ago as being rather more like that of the orang-utan than anything else[15] (and this has been confirmed by a recent study[16]) is nevertheless generally treated in discussion as if it were essentially human.

Questions therefore arise. Do the various postcranial fragments of different australopithecines provide evidence of similarity to man? To what extent, whatever the similarity, are they telling us about biomechanical efficiency for their various biological functions rather than about genetic relationships? Through the initial stimulus and investigations of Professor Lord Zuckerman, through collaboration with him and with Professor E. H. Ashton, Mr T. F. Spence and other colleagues in the Department of Anatomy, University of Birmingham (of which Lord Zuckerman was originally the Head), through collaboration with Professor F. P. Lisowski of the University of Hong Kong, and from studies in my own laboratory in the Department of Anatomy, the University of Chicago, we have used the multivariate morphometric approach to make a series of comparisons between a small number of individual fossil fragments and the equivalent bones both of living men and of a broad range of extant non-human primates[17,18].

The core of the multivariate approach is the following: if we suppose that a single object can be defined by three variables then that object can be represented as a point within a three-dimensional coordinate system. A group of similar objects will then appear as a tightly knit cloud or swarm of points within that three-dimensional space. Other different groups of objects will appear as other clouds or swarms which may or may not overlap with the first. If the original variables defining the objects are not correlated with one another then the original coordinate system will best describe the arrangement of the swarms. If, however, the original variables describing the objects are correlated with one another to some or other degree (the usual situation) then the true relationships among the clouds will best be seen from the vantage point of some other set of coordinate axes. Geometrically, this is the equivalent of

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constructing from one viewpoint a model of the clouds and then rotating the model and scanning it from a new position that gives a view best separating the clouds. If we extrapolate such a three-dimensional geometrical description to an example in which we have taken many variables on many groups then the problem is multi-dimensional and can only be solved algebraically. An equivalent to building the model and rotating it can be carried out through the manipulations of matrix algebra and using a computer[19].

Although multivariate statistical approaches have been used by many workers in different anatomical regions and in different groups of animals and plants for characterising and comparing complex shapes, in our laboratory they are used in a rather special manner for examining postcranial elements. The features used for comparison have been chosen in such a way as to maximise the content of the data that are related to biomechanical function. Studies of several anatomical regions—shoulder, pelvis, ankle, foot, elbow, wrist and hand are now available for extant non-human primate species, for man and for australopithecines.

Structure and function in living primates

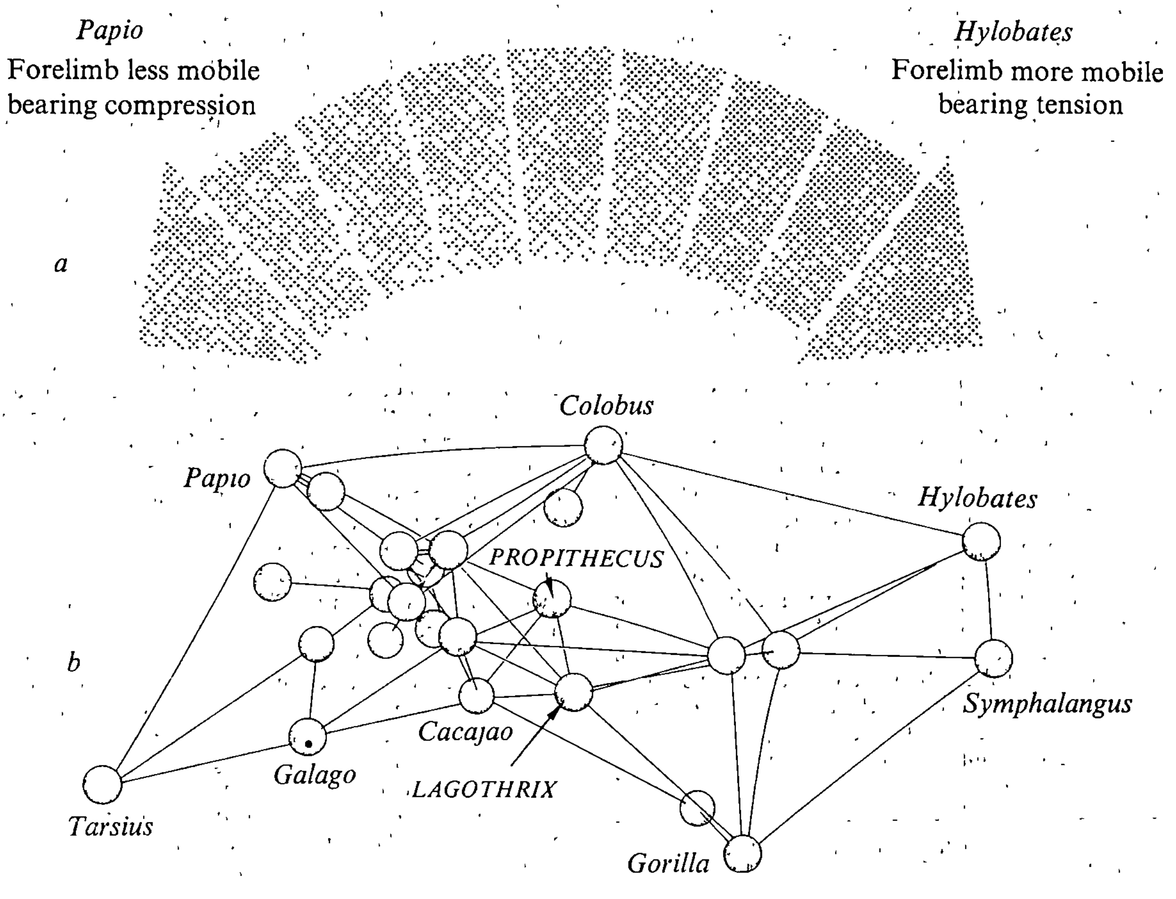
It is thus information about function rather than genetic propinquity that is presumably best presented by such quantitative studies of skeletal fragments. For many of the details of skeletal form are associated with stresses present as a result of the underlying functions of the bones in the life styles of the different animals. Unfortunately we do not know precisely how to assess for this purpose the many different activities that make a life style. It would seem unlikely for instance that posture would be as important as locomotion because the stresses involved, except in specific parts such as tension-bearing ligaments or compression-bearing callosities, would generally be rather small. It is considerably more difficult to assess the relative importance of functions due to rare but life-saving activities and those associated with ordinary locomotion. And very little is known of the impact of small but unremitting cyclical functions, such as heart beat and respiratory rhythm. Nevertheless, it is to a complex "resultant" of these functions that the shape and structure of individual bones seems to be most related. And because it produces the largest forces locomotion will be a major contributor. This seems to be so whether we study the scapula or the pelvis, the forelimb or the hindlimb, the hand or the foot, although it seems that some areas do provide such functional information rather more readily than do others (E. H. Ashton, R. M. Flinn, C.E.O. and T. Spence, unpublished).

It is thus not easy to characterise structurally important aspects of behaviour. For many years[20,21] as well as recently[22,23] this characterisation has been attempted by making locomotor classifications based on the total behaviour of the animals. These classifications have resulted in controversy[24] because it is scarcely possible to group the entire activities of the different primates other than to say that they are all different.

When, however, one wishes to draw associations with the structures of particular anatomical regions there is some value in attempting to classify the functions (very broadly conceived) associated with particular anatomical regions. Our early classifications for the forelimb, hindlimb and vertebral column of primates[25] proved useful by suggesting aspects of morphology that might be closely related to such aspects of behaviour in widely different species, but is proving less valuable as morphological comparisons are attempted among more similar forms[26–28].

Accordingly we have now tried to characterise structurally associated aspects of behaviour as spectra of the

Fig. 1 The band-like spectrum of function in the primate forelimb (*a*) and the model of forelimb structure that can be constructed from a multivariate statistical study of proportions of the forelimb. The model (*b*) is some thirty standard deviation units in its largest dimension. Based on original data from Professor A. H. Schultz. These results are confirmed by studies of other anatomical regions[18].



functions associated with the appropriate anatomical regions. The location of different animals within such spectra are not constant from anatomical region to anatomical region because the functional relationships of different regions are not the same in all species. The form of the functional spectra for different anatomical regions may vary from rather simple two-dimensional analogies to quite complex multi-dimensional star-shaped models. The use of such complex yet broad, diffuse and relatively nonspecific characterisations is forced upon us in the absence of any detailed understanding of the relationship between bone structure and motor behaviour.

The structural patterns found in each anatomical region in the different animals can be characterised in more detail because of the use of quantitative data from the elements of individual animals and because of the use of multivariate statistical and other methods for adding together many different aspects of form for each animal. On these bases we can be much more confident of the existence of continuous structural spectra, though the accidents of evolution may sometimes apparently leave us with "holes" in our spectra.

Once functional and structural spectra are constructed it may be possible to map from the one to the other. The associations may sometimes provide information about the nature of the causal link between function and structure; at other times they may enable us to understand how functions may have evolved even when we have no temporal data but must rely on parallels for the inferences.

How in practice have such ideas worked? Morphometric studies in primates of the form of the scapula, the arm, the forearm, and the overall proportions of the forelimb as a whole, provide, in their generality, a picture of forelimb structure as a rather simple broad band or swathe-like spectrum ranging from species such as baboons and patas monkeys at one extreme to forms such as gibbons and orang-utans at the other. This seems to correlate fairly well with the notion of a broad band or swathe of forelimb function. At one extreme of such a functional spectrum are species (for example, baboons) in which the forelimb tends to be used in quadrupedal locomotion on a two-dimensional substrate (the ground or the large branches of trees) so that the limb typically moves in a two-dimensional antero–posterior arc and bears mainly compressive forces. At the other end of this spectrum are animals (for example, gibbons) in which the limb tends to be used in a raised position, moving in all three dimensions and bearing tensile forces during a rather wider range of movements in the trees where locomotor, postural, foraging and feeding activities are likely to result in such forces. Most primates are intermediate between the two (Fig. 1).

When, however we look towards morphological studies of the pelvis, of the foot and of the overall proportions of the hindlimb as a whole, we find that the distribution of the non-human primates is different from that revealed in the forelimb studies. On the basis of structure they seem to fall into a star-shaped distribution. Functional considerations in the lower limb in these animals place all those species which may be said to be generally quadrupedal towards the nucleus of the star, whereas those primates that are thought to be more highly specialised are those aligned along different rays of the star. The finding of most interest is, of course, that the particular species placed in the nucleus or in any particular arm of the star do not appear to be especially closely related taxonomically; rather do they seem to have similar structural specialisations for functional elements of locomotion that impinge on the hindlimb (Fig. 2).

With a background of such structural–functional associations for several different anatomical areas in living forms, it should be possible to make functional inferences for fossil species. Before we attempt such a procedure, however, let us examine some extant forms in this way. We can then compare the result of the method with the behavioural reality in a way that can never be attempted with fossils.

Two species that may be chosen are the woolly monkey (*Lagothrix*) and sifaka (*Propithecus*). In the various analyses of the shoulder, arm, forearm and forelimb as a whole, these two animals are not very distant from one another, both lying in intermediate positions within the broad band-shaped arrangement that stems from each study (Fig. 1). If the functional–structural associations for these studies have some meaning then we would surmise that, whatever the actual behaviour of these animals, they show some parallel in having mobile forelimbs bearing tensile forces intermediate between the extreme species: gibbons and baboons. Study of the behavioural literature confirms that the woolly monkey and sifaka move in different ways but suggests that the parallels in forelimb function are real. Both animals are well known for a considerable degree of three-dimensional movement of the forelimb, for using the forelimbs above the head in tensed or even sometimes in hanging postures, and for using the hands for drawing in terminal branches during feeding, thus producing tensile forces in the limb. Neither species does these things as much or as well as gibbons, as rarely or as poorly as baboons.

In the various analyses of hindlimb structures (of pelvis, and hindlimb dimensions as a whole), however, these two animals, the woolly monkey and the sifaka, are remarkably distantly placed from one another. Within the star-shaped spectrum that results from each of the hindlimb studies neither species lies in the main nucleus of quadrupedal forms; each lies out on a ray. Each is found in association with other species that have similar biomechanical arrangements in the hindlimb. The woolly monkey is close to gibbons and siamangs which do not leap. The sifaka lies with other indriids that leap a great deal, together with one or two other species (*Hapalemur*, *Lemur catta*) which leap in a similar manner but not as much (Fig. 2).

On the basis of these species therefore, the inference that might be drawn about their positions in both of the functional spectra from their actual loci in the associated morphological spectra is confirmed from what is actually known of their general activities. But the total behaviour and the overall morphology of each living form is unique unto itself. Thus if these total behaviours and overall morphologies were not known for the woolly monkey and the sifaka—if they were fossils—they could still be described through similarities and differences to other living forms Most species are, thus, describable as mosaics of the parts of other species. Though there is little point in characterising living species as structural mosaics in this way it is a useful exercise in assessing fragments of fossil species. For although it is unlikely that any particular fossil moved in exactly the same way as any single living species, consideration of the fossil as a mosaic of structures may provide some notion as to what it may have done.

Place of man among non-human primates

In all of the studies presented above, the discussion centres around the non-human primates. Thus, before passing to the examination of fossils, it is of interest to view the position of man. Previous understanding of primate taxonomy and evolution through comparative anatomy suggests that man falls relatively close to the non-human primates in a locus related to the chimpanzee and gorilla (although it is true that traditional taxonomy places the African great apes with the Asiatic great ape, as a little separate from man[28]) In more recent years data relating to the anatomy of molecules also confirm the overall relationship of man

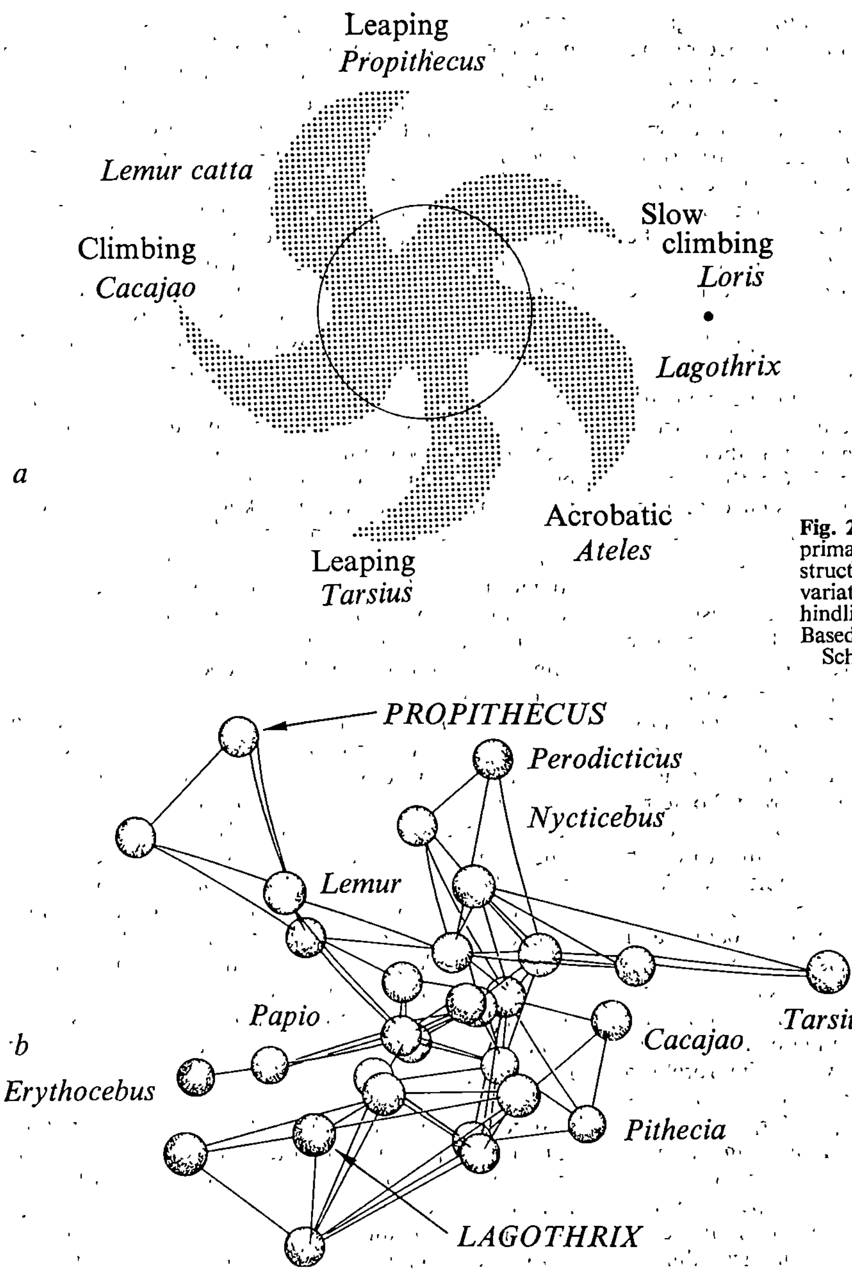


Fig. 2. The star-like spectrum of function in the primate hindlimb (*a*) and the model (*b*) of hindlimb structure that can be constructed from a multivariate statistical study of proportions of the hindlimb. The model is some 24 s.d. in radius. Based on original data from Professor A. H. Schultz and confirmed by other studies[18].

to the non-human primates (although it is of interest that, in the details of these more recent studies, the chimpanzee and gorilla are placed unequivocally closer to man with the Asiatic orang-utan somewhat more distant[30]).

The results of the current studies, in giving more information about structurally relevant aspects of behaviour than about genetic propinquity, do not present these classical pictures; man is not, in these studies, especially close to the primates as a whole or to the chimpanzee in particular. Nor, however, can man be described as a mosaic of other forms. In almost all studies man lies quite separately from the spectra of non-human species although he is, of course, somewhat closer to particular species (for example, sifaka, saki monkey, orang-utan, chimpanzee) than to most others (Fig. 3).

This uniqueness for man does not contradict the findings from traditional morphology or from the molecular studies referenced above. This uniqueness presumably arises from his having a totally different behavioural repertoire from any other primates, and therefore unique functions in different anatomical regions. The lack of a mosaic description presumably results because functional parallels are not available from other living primates. Although all primates can use their forelimbs for manipulation, communication, feeding, and so on, the overall functions of the human forelimb are different from those of other primates because man does not use this member for locomotion. Although almost all primates can walk on two legs, the functions of the human hindlimb are uniquely different because only man does this habitually and also, and perhaps especially, because only man is utterly unable to use the hindlimb in quadrupedal locomotion.

The findings for man provide, therefore, not only additional important information necessary for studying fossils that may be thought to be close to human, but also further confirmation of the functional, more than any other biological, such as genetic, emphasis to these particular morphological results.

The functional status of australopithecines

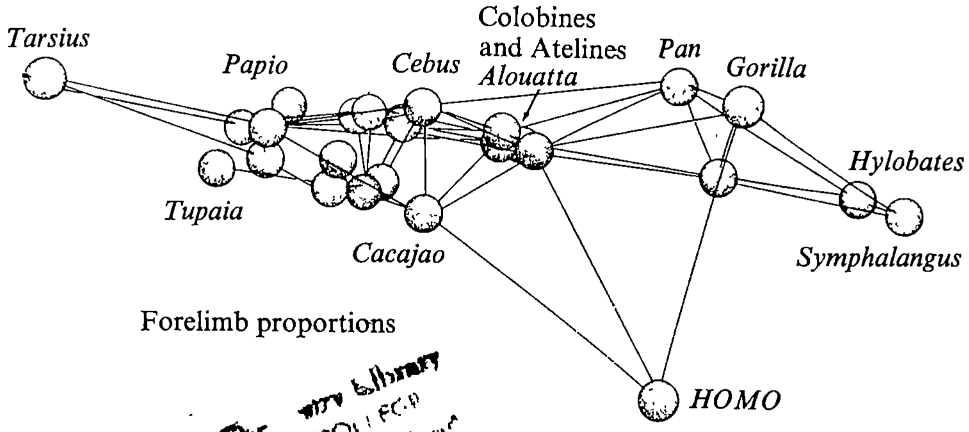
The third and final set of comparisons contains the ones in which we have most interest: those between the various fossil genera that are generally believed to be associated with the human lineage.

Fossils such as Neanderthals need scarcely be considered. A great deal is known about their anatomy and we may assume that they are very similar to modern man; surely, for instance, they were bipeds. It turns out that the only postcranial anatomical region for which we have fragments that have been compared by multivariate morphometrics is a particular ankle bone—the talus. These studies show unequivocally that several different Neanderthalers (Spy, Skhūl[10,31,32] and Kiik-Koba[31,32]) are similar to man and quite different from non-human primates.

A much earlier series of fossils, the talus bones of Miocene apes, have also been studied in this way. These are reminiscent more of gibbons and siamangs than of other living primates. Their next closest morphological similarities are with orang-utans[31,32]. Thus these particular tali may have belonged to the feet of small, relatively arboreal, at least somewhat acrobatic, seldom-leaping creatures. They are certainly not like those of man, of present-day African apes or Old World monkeys. These fragments do not offer any particular controversy although it would be of great interest if access to the relatively full skeletal remains of *Oreopithecus* could be obtained so that many different anatomical areas could be examined.

Between the very early Miocene apes and ancient man is the tantalising set of fossils known as *Australopithecus*. Although most workers feel that the overall position of these fossils is adequately fixed, with a taxonomic label as clearly Hominidae, an evolutionary label as on the line to man or very close to it, and a functional label as a human type of biped and tool user and maker, the postcranial fragments available have not been fully studied. Most conclusions so far mirror the conventional ideas obtained from skulls and teeth. But our current studies are providing rather different ideas.

In the multivariate investigations reported here, the various australopithecine fossils are usually quite different from both man and the African apes (except in those features which are common to all hominoids or to all anthropoids). The fossils can be described by mosaic characteristics (Table 1). Viewed as a genus they are a mosaic of features unique to themselves and features bearing some resemblances to those of the orang-utan. As with the results for the woolly monkey and sifaka this mosaic



Forelimb proportions

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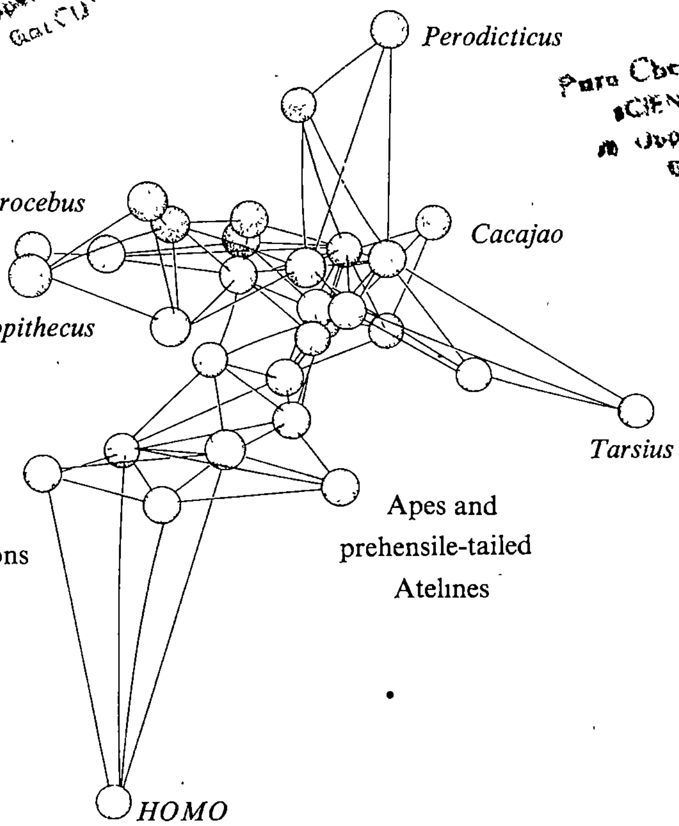

Hindlimb proportions

Fig. 3 The models of the structure and function of the fore- and hindlimbs rotated to show the position of man[18].

Table 1 Summary of results of multivariate studies of australopithecine fragments

Generally recognised resemblances with man	Resemblances in current studies Orang-utan	Resemblances in current studies Unique
Scapula	+	
Clavicle	+	
Humerus*		+
Metacarpal*		+
Phalanges*	+	
Pelvis		+
Talus	+	
Toe phalanx		+

*These anatomical areas thought to be somewhat less like man than the remainder.

condition presumably does not speak to genetic relationships. Such resemblances as there are between these fossils and the orang-utan must result from functional parallels. And because similarities with the orang-utan are only in some anatomical regions and not in others, because the overall comparison is mosaic in nature, it is clear that the actual overall mode of locomotion of the orang-utan today is not the model for these creatures. If the fossils have ankle, hand and shoulder bones patterned somewhat after those of the orang-utan then we can only surmise that perhaps, as the orang-utan, the fossils had ankles, hands and shoulders adapted for climbing. Because they have pelves that have articular relationships parallel to those of man, we may guess that perhaps they stood and moved upright with a vertical load distribution But because the muscular features of the pelvis are positioned in a way more like those of the great apes, we must estimate that their muscular arrangements were therefore rather similar to those associated with climbing and perhaps quadrupedalism. They have relatively small articular surfaces in the hindlimb as compared with the forelimb, which parallels most closely the orang-utan, and contrasts markedly with man who has big articular surfaces in the leg compared with the arm. They may have been bipedal in a way that is no longer seen, but have retained abilities for climbing, and perhaps minor arboreal acrobatics such as might be found in an intermediately sized ape-like creature. If these estimates are true, then the possibility that any of the australopithecines is a direct part of human ancestry recedes; the study of their possible posture and locomotion becomes a fascinating adventure in its own right[17,18].

Incidentally it must be pointed out that this mosaic result for the australopithecines is not an artefact of grouping the various australopithecine specimens of possibly different species into a single genus. First, the difference between the species, (if they truly exist) is considerably less than between the species in many extant genera. Second, in those cases where we have postcranial specimens of more than one australopithecine there is not usually found any great difference The talus bones from Olduvai (*A. africanus* or "*H. habilis*") and from Kromdraai (*A robustus*) are scarcely distinguishable from one another and fit in well with a whole host of non-human Pleistocene tali from Africa[32]. The humeral fragments, though distinguishable from each other, are quite different from present-day man or any living apes The various femoral remains of both robust and gracile forms show the small heads and inclined femoral necks that might be expected in animals capable of quadrupedal activities.

Independent supporting evidence

What is the possibility that this radical re-interpretation of the postcranial elements of these fossils may be correct? Three other pieces of evidence support these ideas and are independent of those assembled in our laboratory.

One comes from the recent finding at East Rudolf by Richard Leakey[33] of a new talus lying between layers that have been dated at between 1.5 and 2.6 Myr by argon isotope techniques. This specimen is thus as old as, if not appreciably older, than the australopithecine talus from Olduvai noted earlier in this article. Yet this new specimen seems to be much more like that of man as far as can be judged from the published picture and descriptions. Further description and examination using multivariate statistical methods[34] confirms that it is indeed similar to modern man and unlike the australopithecine specimens. The second indication comes from the finding, again by Leakey[35], of a skull dated at almost 3 Myr (certainly more than 2 Myr) and with an endocranial volume of nearly 800 cm^3 and possibly more (compare with *Homo erectus* at 900 cm^3); this may be contrasted with the australopithecines, the most recent of which may be less than three-quarters of a million years old and the cranial capacities of which are often only about 450 cm^3, smaller than of a large gorilla disregarding difference due to overall bodily size. The third piece of evidence comes from a find made some years ago —a fragment of arm bone perhaps four or more million years old from Kanapoi. This has already been shown to be very similar to that of modern man[36], and some of our demonstrations clearly support that contention[18].

Thus, unless tali, crania and humeri evolved through cycles such that they were more like man at early stages, less like him at later australopithecine stages, and more like him again at an even later human stage, then the australopithecines must have lain on an evolutionary side branch for a long time.

These pieces of evidence may also mean that perhaps as long as five million years ago or even longer there may have been creatures living that were generally somewhat similar to *Homo erectus* and therefore classifiable as man in a way that we must now deny to *Australopithecus* (whether named "*H. habilis*", "*H. africanus*" or whatever else). The removal of the different members of this small-brained curious genus, *Australopithecus,* into several parallel side lines distinct from the direct line to modern man would make it easier to accept the findings described here And this re-assessment is to be seen in the revision by at least one authority[37] of the age of the Taung australopithecine, so that it postdates the arrival of true *Homo* in Southern Africa according to the conventional notion. At the same time the possible age of a human–non-human link is rapidly being increased; on one assessment, which originally dated a chimpanzee–human common ancestor at around 3 Myr[38], a revised estimate gives a date of six or even 10 Myr[39] while many other authorities[40,42] maintain, of course, that much longer periods characterised this particular evolutionary divergence.

The existence of individuals somewhat like *Homo* (in the very few features available for comparison) this much earlier has further, broader implications. The length of time available for the psycho-social evolution of *Homo* may well have been almost an order of magnitude greater than had been believed. Knowing as we do the enormous speed of psycho-social evolution by comparison with biological evolution, then perhaps more of modern man's character should be attributed to psycho-social, and less to biological, evolution than some would have us suppose.

Thus the cruel and sophisticated expression of man's aggression towards his own species may be less dependent on a previous animal base and more dependent on the relatively longer span of psycho-social evolutionary time. The extreme intricacies of human culture may likewise reflect considerably less on a previous basic behaviour shared with non-human primates and somewhat more on psycho-social evolution. Human intellectual prowess may have had as much as ten times more time to increase from a level comparable to that of some non-human stage of behaviour than we have previously realised.

More evidence of earlier forms that are more like man than australopithecines will be found even though these latter must also have existed at earlier periods in time. Presumably more fossil remnants will be discovered outside Africa because over such a long time period, human or prehuman populations must have existed in other places, with migrations, and with multiple evolutionary lines.

We may have to accept that human bipedality is far older than previously guessed. With that may go a far more ancient free forelimb with its associated abilities for tool using and tool making. We may have to accept that the australopithecine form (or forms) of locomotion, tool using and tool making may be merely one (or more) unsuccessful evolutionary experiments existing in parallel with those of man. We may well have to accept that it is rather unlikely that any of the australopithecines, including "*Homo habilis*" and "*Homo africanus*" can have had any direct phylogenetic link with the genus *Homo* except perhaps at earlier times.

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articles

Age of KBS Tuff in Koobi Fora Formation, East Rudolf, Kenya

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Conventional K–Ar dates on pumice from the KBS Tuff horizon in the Koobi Fora Formation at East Rudolf, Kenya, distinguish two tuff units. Samples from area 10 and area 105 give an age of 1.60±0.05 Myr whereas those from area 131 give 1.82±0.04 Myr. Both ages differ from the previously published age of 2.61±0.26 Myr obtained by $^{40}Ar/^{39}Ar$ technique.

PLIO-PLEISTOCENE lacustrine and fluvial strata exposed along the east side of Lake Rudolf, Kenya, now known as 'East Rudolf', have been under study since 1969 when Behrensmeyer began geological investigations, which led to the identification of the KBS tuff in area 105 (Fig. 1)[1-4]. These strata, resting uncomformably on late Miocene and Pliocene volcanics, are notable for their extraordinarily rich assemblages of fossil vertebrates, which include a large number of hominid remains. Artefacts also occur in some places[5-8].

Interspersed in the stratigraphic sequence are several rhyolitic tuffs, mostly reworked, which have served as marker beds and for calibration of the fossil vertebrates and hominid artefacts by potassium–argon dating[9-11] and in one instance by fission-track dating[12]. Dates on tuffs within the Koobi Fora Formation range from 3.18 Myr for the Tulu Bor Tuff in the Lower Member, to 1.22 Myr for the Chari Tuff and the correlated Karari Tuff in the upper part of the Formation. The KBS Tuff from the type locality in area 105 was assigned an age of 2.61 Myr, although subsequent age determinations resulted in a broad scatter of dates, both older and younger than this value[10,11]. Contamination by ancient bed rock material during the reworking of the tuffs was suggested to account for the anomalously old dates, whereas subsequent alteration, 'overprinting', of the pumice fragments used for dating, by alkaline-rich and possibly heated ground water may explain the anomalously young dates by partial loss of radiogenic argon.

Using the incremental heating technique of $^{40}Ar/^{39}Ar$ dating, Fitch and Miller believed they could resolve these overprinting events by analysis and interpretation of age spectra, and

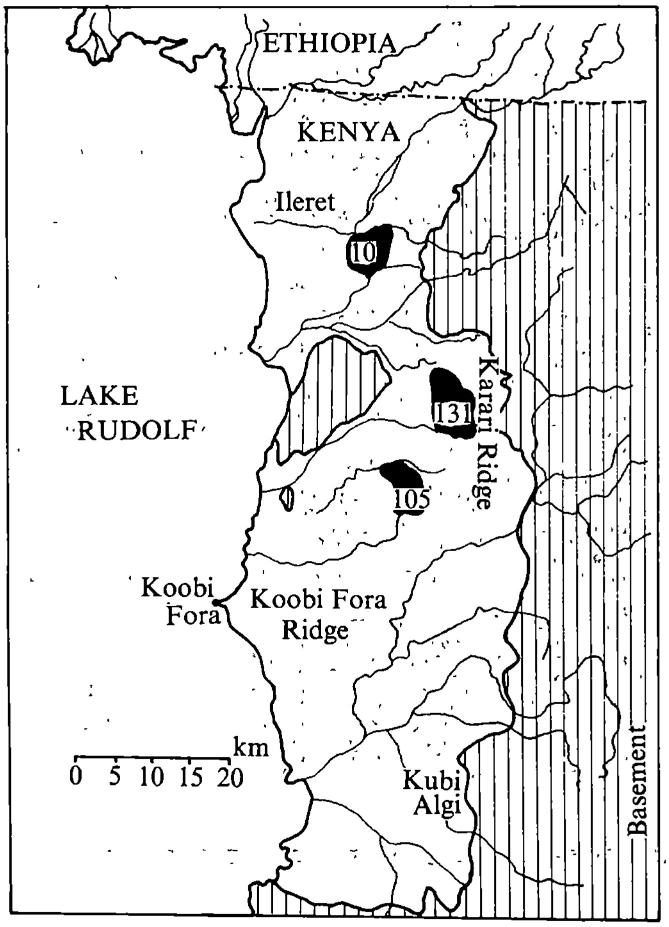

Fig. 1 Map of the East Rudolf region, showing the sampling areas.

arrived at the 'best apparent age' for the KBS Tuff of 2.61 ±0.26 Myr (ref. 10).

When some palaeontologists compared fauna associated with the KBS Tuff in East Rudolf with those of other, supposedly well calibrated localities, the reliability of the date of 2.61 Myr for the KBS was questioned. Although Maglio found that the morphology of elephant fossils fit with a 2.5 Myr date[7], Cooke and Maglio, in 1972, pointed out that fossil pigs from below the KBS Tuff horizon at East Rudolf seemed to correlate best with those from beds dating close to 2 Myr in the Omo River area to the north in Ethiopia[13–16]. Because of the great interest in the dating and the question of reliability raised by the scatter of results from the KBS Tuff, we repeated the dating using the conventional method of K–Ar dating.

It is argued that the conventional K–Ar method is unable to evaluate results obtained by the ^{40}Ar/^{39}Ar method, because it assumes that all non-radiogenic argon in the sample has the same relative abundance as in the atmosphere, an assumption demonstrably wrong in some cases. Although this may be true for a single determination, multiple K–Ar determinations made on several samples of a tuff, and on more than one type of material can be plotted on an isochron diagram to show the isotopic composition of the non-radiogenic argon in the tuff.

With respect to the ^{40}Ar/^{39}Ar dating method, Dalrymple and Lanphere have pointed out (personal communication) that even after 5 yr of research with this method they occasionally obtain anomalous ages for geologically young rocks (late Tertiary and younger) which they are unable to explain. Furthermore, the interpretation of the ^{40}Ar/^{39}Ar isochrons that yield multiple apparent ages from a single phase, such as Fitch and Miller have obtained on KBS sanidines, is uncertain. Their interpretations rely on oversimplified and unproved models for the diffusion of argon in solids, both in nature and in the laboratory procedure necessary to make an age determination. For this reason we feel that their results, even when they are reproducible to high precision, may be an artefact of experimental procedure, and thus not geologically meaningful.

Analytical procedure

During the summer of 1974 samples of pumice were collected from outcrops now classified as the KBS Tuff in three localities. One additional pumice sample from the eastern part of area 105 had been collected by Lucas previously for geochemical analysis during palaeomagnetic sampling in the summer of 1973. These samples were subsequently prepared for dating, using selected pumice fragments which were first examined both by eye and in thin section. Most sections showed the principal minerals, sodic sanidine and pyroxene, to be fresh, although the enclosing vesicular glass ranged from highly altered in most specimens to fresh in only three. Calcite filled most vesicles, although zeolite was present in some.

Sanidine samples were separated from the glass with bromoform after the pumice had been cleaned, crushed and sieved. The concentrates were then treated with 1 N HCl to remove calcite, and 5 % HF for 1 min to remove adhering clay. Splits of these samples were then analysed for argon, using

Fig. 2 Isochrons for KBS Tuff. *a*, ^{40}Ar/^{36}Ar against ^{40}K/^{36}Ar; *b*, ^{40}Ar against %K. ●, Data from area 131; ▲, areas 105 and 10. Plots obtained by least-squares fitting. The ratio of ^{40}Ar/^{36}Ar in air is 296.0.

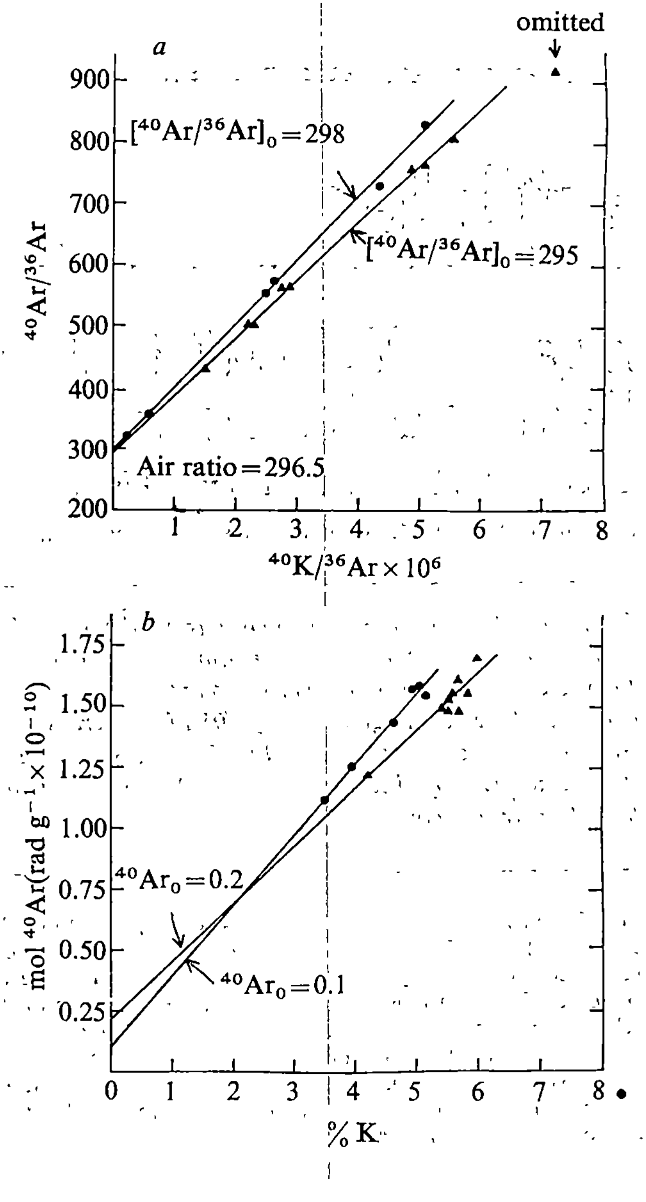

Table 1 KBS Tuff ages (Myr)

Area 105		Area 10		Area 131	
1.65 (S) 1.50 (S)	0001	1.54 (S) 1.60 (S)	0002	1.84 (G) 1.83 (S) 1.81 (S)	0001
1.62 (S) 1.56 (S) 1.62 (S)	0002	Mean of 2 = 1.57		1.83 (G) 1.85 (S) 1.73 (S)	0002
1.68 (G) 1.61 (S)	Lucas			Mean (±s.d.) of 6 = 1.82±0.04 Myr	
Mean of 7 = 1.61					
Mean (±s.d.) of 9 from 105 and 10 = 1.60±0.05 Myr					

G, glass; S, sanidine.
Samples with detrital contamination have been omitted.

procedures described by Dalrymple and Lanphere[17], and for potassium on a Zeiss PF5 flame photometer following dissolution of samples in HF and neutralisation.

The first dates (KA 2816, KA 2817, KA 2818, and later KA 2851) obtained from samples prepared in the manner described showed no agreement (see Table 3). Microscopic examination of the remaining concentrated crystals revealed numerous yellow grains of rounded and altered K-spar, clearly detrital in origin and probably derived from ancient bedrock. These grains had been tightly cemented on the convoluted surfaces of the rounded pumice, and had escaped detection in spite of careful cleaning. Subsequent work proved that it was virtually impossible to exclude these detrital sand grains completely when mineral separations were made using heavy liquids. To avoid this problem, we carefully crushed individual pumices in a hand mortar, extracting the large phenocrysts with tweezers under a magnifying glass or binocular microscope. The phenocrysts were then treated with HF, crushed again, and put through a magnetic separator to remove included and adhering pyroxene grains.

Only three specimens 131-0001, 131-0002, and the Lucas sample from area 105 had glass fresh enough for both sanidine and glass age determinations to test for possible effects of overprinting. Since sanidines and glass have markedly different physical and chemical properties, it is highly unlikely that any substantial overprinting event would produce an exactly proportional loss of argon, such that their apparent ages would remain the same. It is equally unlikely that several sanidines from widely separated samples would yield identical apparent ages, had overprinting occurred, as the intensity and duration of these events are not everywhere uniform. Thus, when concordant dates are obtained on two different phases from the same sample which also closely agree with single dates on other samples the probability becomes increasingly large that these dates are an accurate estimate of the true age of their formation.

Results

Three different pumice clasts from the KBS Tuff at area 105 and one from area 10 (Ileret) give an average age of 1.60±0.05 Myr for nine analyses, while two separate pumice clasts from the tuff in area 131 (Karari Ridge), hitherto correlated with the type KBS in area 105, give an average age of 1.82±0.04 Myr on six analyses (Table 1).

The narrow range of dates and high precision obtained on all samples, once sufficient care was taken in sample preparation, we believe distinguish the tuff in area 131 from the KBS in area 105 and area 10. This same distinction is seen by the comparison of average K contents for the uncontaminated sanidine separates used for age determination. The sanidines from area 105 and area 10 average 5.6% with a range of 5.36–5.94%, whereas those from area 131 average 4.90% with a range of 4.60–5.11%.

Table 2 Concentrations of niobium, zirconium, yttrium and rubidium in pumice

Locality	Sample number	Nb	Zr	Y	Rb
		Concentrations (p.p.m.)			
KBS 105	ER72-105-0004	500	757	144	98
	Lucus-TC-A	502	746	144	101
	Lucus-TC-B	503	735	139	103
KBS 131	ER74-131-0001	333	1,357	121	87
	ER74-131-0002	926	1,664	303	146

Nb and Y were analysed using BCR-1 as standard. Zr and Rb were analysed using G-2 as standard.

Table 3 KBS analytical data

KA no.	Sample no.	Material dated	%K	%atmospheric ^{40}Ar	mol ^{40}Ar rad g^{-1} $\times 10^{-11}$	Age × 10^{6} yr	Remarks
2816	ER74-010-0002	Sanidine	5.167±0.013	17.8	6.203	6.90±005	detrital contamination
2817	ER74-105-0002	Sanidine	5.035	37.8	1.755	2.01±0.03	detrital contamination
2818	ER74-105-0002	Sanidine	5.006	41.2	2.089	2.40±0.03	detrital contamination
2823	ER74-131-0001	Sanidine	4.808	40.6	1.807	2.16±0.03	detrital contamination
2836	ER74-131-0001	Sanidine	4.993±0.019	35.7	1.584	1.83±0.02	hand picked crystals, HF 2 min
2837	ER74-131-0001	Glass	3.922±0.020	92.5	1.252	1.84±0.07	heavy liquid separation, HF 2 min
2841	ER74-131-0002	Sanidine	4.896±0.031	51.6	1.569	1.85±0.03	hand picked crystals, HF 2 min
2842	ER74-131-0002	Sanidine	5.110±0.045	40.6	1.539	1.73±0.03	hand picked crystals, coarser fraction than KA 2841
2843	ER74-131-0002	Glass	3.484±0.0002	82.9	1.105	1.83±0.03	heavy liquid separation, HF 2 min
2850	ER74-105-0001	Sanidine	5.665±0.040	32.2	1.479	1.50±0.02	hand picked crystals, HF 2 min
2851	ER74-105-0001	Sanidine	~5.94	31.0	2.605	2.53±0.02	detrital contamination
2851R	ER74-105-0001	Sanidine	5.936±0.007	39.1	1.698	1.65±0.02	same as KA 2851 with detrital grains picked out by hand
2852	ER74-010-0002	Sanidine	5.785±0.004	68.9	1.545	1.54±0.02	hand picked crystals, HF 2 min
2854	Lucas-105-East	Glass	4.171±0.0001	52.7	1.217	1.68±0.01	heavy liquid separation HF 10 min to remove zeolite
2855	ER74-105-0002	Sanidine	5.468±0.024	58.9	1.481	1.56±0.02	hand picked crystals, HF 5 min
2856	Lucas-105-East	Sanidine	5.487±0.020	36.7	1.529	1.61±0.02	hand picked crystals, HF 5 min
2857	ER74-105-0001	Sanidine	5.543±0.021	52.5	1.558	1.62±0.02	hand picked crystals, HF 5 min
2859	ER74-105-0002	Sanidine	5.643±0.034	58.7	1.613	1.65±0.02	hand picked crystals, HF 5 min
2861	ER74-010-0002	Sanidine	5.359±0.0009	38.8	1.485	1.60±0.01	hand picked crystals, HF 5 min
2869	ER74-131-0001	Sanidine	4.598±0.0002	53.4	1.441	1.81±0.02	hand picked crystals, HF 5 min

$^{40}K/K = 1.18 \times 10^{-4}$; $^{40}K_{\lambda} = 5.480 \times 10^{-10}\ yr^{-1}$; $^{40}K_{\lambda\beta} = 4.905 \times 10^{-10}\ yr^{-1}$; $^{40}K_{\lambda e} = 0.575 \times 10^{-10}\ yr^{-1}$.

X-ray fluorescence analyses[18] show the distinctive Nb, Zr, Y, and Rb contents of pumice glass from the two tuff units (Table 2). Sample 0001 from area 131 and those from area 105 are, however, similar in appearance, being composed of dark brown glass with open vesicles enclosing large euhedral sanidine phenocrysts, whereas sample 0002 from area 131 is composed of a grey glass with elongated tubular vesicles enclosing less abundant sanidine phenocrysts. These pumices with fresh glass are rare compared with those which are completely altered to clay minerals and zeolites.

Although the difference between KBS–105 and KBS–131 is clear, the data for the samples from area 131 show that two different pumices have been sampled. Even though they are of the same age, they may be derived from different eruptive centres. This introduces the possibility that older pumices may also be present in the KBS tuff horizon which could account for the 2.61 Myr date reported by Fitch and Miller. For this reason, all dates obtained on pumices should be considered as maximum ages for the tuff itself.

The high precision and concordancy of dates obtained on all samples, in spite of the fact they ranged from fresh to highly altered, indicates to us that none has lost significant argon after deposition.

This conclusion is further supported by two isochron plots (Fig. 2) (and see also ref. 19). The ^{40}Ar/^{36}Ar against ^{40}K/^{36}Ar isochron shows that the non-radiogenic argon component in both age groups is nearly the assumed atmospheric composition. The ^{40}Ar against % K isochron indicates a slight excess ^{40}Ar component for each group, which would make the apparent ages too old. The narrow range of potassium in this isochron, however, makes the error in extrapolation large; but the fact that a positive rather than negative ^{40}Ar intercept results, argues against argon loss for either sample group.

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Statistical mechanics and topology of polymer chains

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The statistical–mechanical treatment of closed polymer chains based on algebraic topology is proposed. Using the Monte-Carlo method numerical results were obtained for the probability of knot formation during random closing of polymer chains of different length. For very rigid chains such as DNA double helix the probability of knot formation is rather great. Topological restrictions in a system of two polymer chains are shown to lead to a specific topological interaction between them.

THE detailed statistical–mechanical theory of polymer chains[1–4] may be rigorously applied only in the case of linear, unclosed polymer chains. Interest has been shown in the particular case of closed polymer molecules, connected on the one hand, with successful synthesis of a new type of chemical compound, catenanes, consisting of two topologically linked polymer molecules[5]. On the other hand, closed circular DNA molecules are widely distributed in nature. DNA may be found in linear as well as in closed circular form at different stages within the cell[6,7]. In some cases, two or more DNA molecules form catenanes.

One would expect the statistical–mechanical properties of closed polymer molecules to differ considerably from those of linear molecules. This difference originates from the fact that in the former case a topological state formed during closing of the chain must remain unchanged in the course of any conformational transformations of the molecule. This problem is closely related to that of computing the probability of formation of a given topological state by a polymer chain while closing.

Different topological aspects of polymer statistical mechanics have been discussed[8–16], but a solution to the problems of statistical mechanics of closed polymer chains has not been worked out. The major difficulty arises in the formulation of an algorithm to distinguish the different topological states of the chain. In other words, one has to be able to reduce a given entangled polymer coil formed by a closed chain to its simplest form for which the subsequent disentanglement is not possible (Fig. 1). Crippen[15] has attempted to solve this problem in a more direct way, by developing an algorithm which progressively decreases the number of self-intersections of the chain on its projection. Crippen's algorithm has, however, proved ineffective. Edwards[10] has proposed an integral invariant of a knot but this invariant has been shown[17] to be incorrect.

We have proposed[17,18] the use of the results of algebraic topology, in which highly effective invariants of knots and links were developed, and the generation of closed chains on a lattice by the Monte-Carlo method. One may then calculate statistical–mechanical properties of isolated chains of different length, and of a system of two closed polymer chains. We present here the results of such calculations. Details of the theory of knots and links are given in refs 19–21.

Types of knots and links

Knots are classified according to the minimum number of intersections on their projections (Fig. 2 shows knots with less than six intersections). A complete table of all 84 knots having less than ten intersections is given in ref. 21.

A topological invariant best suited to our purposes is the Alexander polynomial, and the values, $\Delta(t)$, corresponding to simplest knots, are given in Fig. 2. For an unknotted closed chain (trivial knot), $\Delta(t) = 1$.

Note that any two chains which are equivalent topologically have been shown to have the same Alexander polynomial[20]. Computation of this value thus solves in principle the problem of reduction of a given arbitrary entangled polymer coil to its simplest form (Fig. 1).

We have simplified computation of the polynomial to a computer algorithm[17]. Note that some degeneracy exists for the invariant under consideration, that is, two topologically different knots may have the same Alexander polynomial. Such coincidences, however, are extremely rare. Specifically the simplest non-trivial knot having $\Delta(t) = 1$ contains eleven intersections on its projection (for example, ref. 17). In the framework of this paper such degeneracy may be considered negligible.

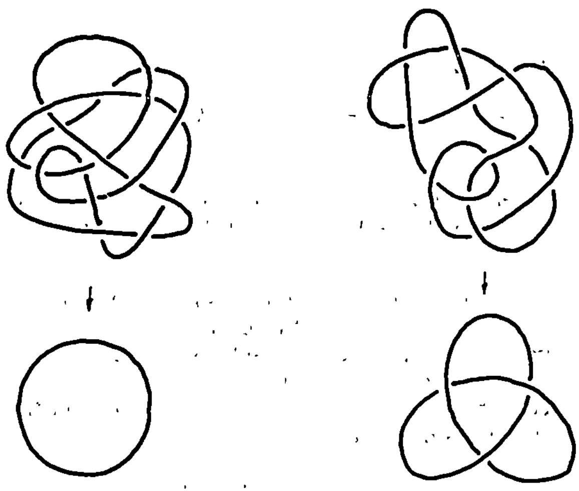

Fig. 1

Links may be classified in much the same way as knots (for example, Fig. 3 shows four of the simplest links). The Alexander polynomial in this case, represents a function of two variables $\Delta(s; t)$. Examples of this polynomial are shown in Fig. 3. For two closed chains not linked with each other $\Delta(s,t) = 0$.

Methods of calculation

To calculate the statistical–mechanical properties of closed polymer chains the Monte-Carlo method was used. The polymer chains were simulated by the paths of random self-avoiding walks on a three-dimensional body-centred lattice. To obtain only closed chains an effective algorithm[17] was used. Note if self-intersections of the chain are excluded in Monte-Carlo calculations, one must calculate the statistical weight for each chain when computing the probabilities of different states of the chain[17,22,23].

Calculations have been made for two models of a polymer chain. The first model corresponds to a 'thick' or flexible polymer chain for which the Kuhn segment length is close to its thickness. Such a chain has been generated directly by self-avoiding random walks on the lattice. The second model corresponds to a 'thin' or very rigid chain for which the Kuhn segment length is much greater than its thickness. To obtain such a chain we allowed the end of the generating path to reach a previously occupied node of the lattice. But each time such an intersection occurred the lattice as a whole was shifted along a randomly chosen direction for a very small distance compared with the period of the lattice. The random walks were continued on this new displaced lattice. Such chain generation guarantees the loss of a true self-intersection, at the same time enabling any two segments far apart on the chain to pass very close to each other in space. By contrast, in the case of the 'thick' polymer chain the most distant segments are not permitted to approach one another closer than the (approximate) period of the lattice.

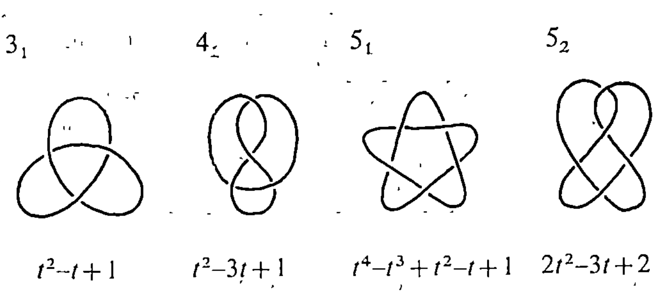

Fig. 2 Simplest non-trivial knots and their Alexander polynomials.

Using one of the methods outlined above a closed non-self-intersecting chain was generated, and its corresponding statistical weight calculated. Each chain was classified as to its specific topological type by calculating its Alexander polynomial. The probability of formation of a particular topological state as a result of random closing of a chain was computed as a sum of statistical weights of chains belonging to a given topological type divided by the total sum of statistical weights of all generated chains.

When considering topological interaction of two closed chains only the model of the extremely 'thin' chain was considered. First, two chains of the same length were generated independently in this case, and were brought closer together, the distance between the centres of mass of the chains having a value R. This was achieved by simple parallel displacement without any deformation of the chain; during such displacement the chains were permitted to penetrate one another freely. When displacement was finished the system formed was classified into its specific topological state by calculating its Alexander polynomial. The probability of formation of a particular topological state was computed as above for the case of a single chain.

Probability of knot formation

What is the fraction of chains of a given length L (number of Kuhn segments) expected to form a trivial knot or a knot of particular type (see Fig. 2) after random closing? Our calculations have shown that this probability depends on the flexibility

Fig. 3 Simplest linkages of two chains and their Alexander polynomials.

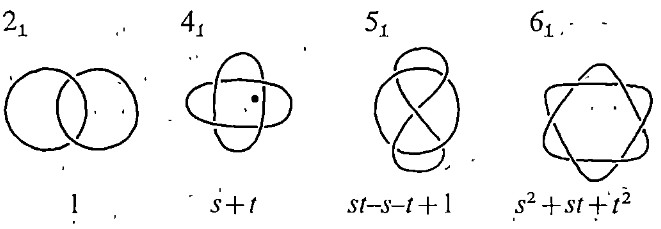

Table 1 Probability of knot formation in the model of thick (or flexible) chain

Chain length (L)	No. of chains inspected	No. of knotted chains	Probability of knot formation
50	49,000	55	$(0.5\pm0.2)\times10^{-4}$
100	13,000	98	$(1.6\pm0.8)\times10^{-4}$

of the polymer chain. For very flexible (or thick) chains for which the segment thickness is close to its length the probability of knot formation is extremely small even for chains containing as many as 100 segments (Table 1). In calculating the probability of knot formation for the model of thick chains, taking into account the correct statistical weights of different paths is important. This may be attributed to the greater compactness of a knotted chain compared with an unknotted one; as a result, the former has, on average, a much smaller statistical weight. Thus, a simple division of the number of knotted chains by the total number of generated chains leads to an incorrect (overestimated) value of the probability of knot formation (Table 1).

In contrast, the probability of knot formation for the model of a rigid (or thin) chain having segment length much greater than its thickness is quite appreciable. Figure 4 shows the calculated dependence of the probability of formation of a non-trivial knot during random closing of a thin polymer chain on its length (in terms of number of segments), L. A chain containing 150 segments has to form a knot in approximately 40% of all cases. Obviously, different knots are formed with different probabilities (Table 2). If short chains mainly form simple knots (trefoil, type 3_1) the fraction of trefoils decreases with increase in chain length, and for $L=150$ such chains amount to only 40% of the total number of knotted chains.

Topological interaction between two polymer chains

Topological restrictions arising in the system of two closed polymer chains lead to a specific topological interaction between them. Let us consider two ideal (infinitely thin) polymer chains having no excluded volume effects. This means that the single effect of interaction between chains, and between different segments of the same chain, consists of the elimination of passages of one segment through another. If both chains are unclosed, a state of one chain will not depend on the state of another; thus, there will be no interaction between them. (We are considering only equilibrium properties of polymer chains. Elimination of passages of one segment through another in unclosed chains should lead to some kinetic effects, and these may be considerable for sufficiently long chains. Such kinetic problems, however, are beyond the scope of our paper.) The closing of one of the chains will not change the situation. But as soon as we close both chains the situation changes drastically. Indeed, the closing of both chains converts the system to some particular topological state (the linkage of some type, Fig. 3) which has to remain unchanged in the course of any subsequent deformations of the chains. This topological restriction decreases the conformational entropy of the system. As a result, free energy of the system of two closed polymer chains depends on its topological state and on the distance between the chains.

The number of conformations of a pair of chains belonging to a specific topological state is the total number of conformations of two isolated closed chains multiplied by the probability of formation of a given topological state during random closing of two chains.

We have restricted ourselves to the simplest case of statistical-mechanical properties of two unlinked closed polymer chains. We need to know therefore only the probability P_0 of formation of unlinked state during random closing of two chains as a function of the distance R between their centres of mass.

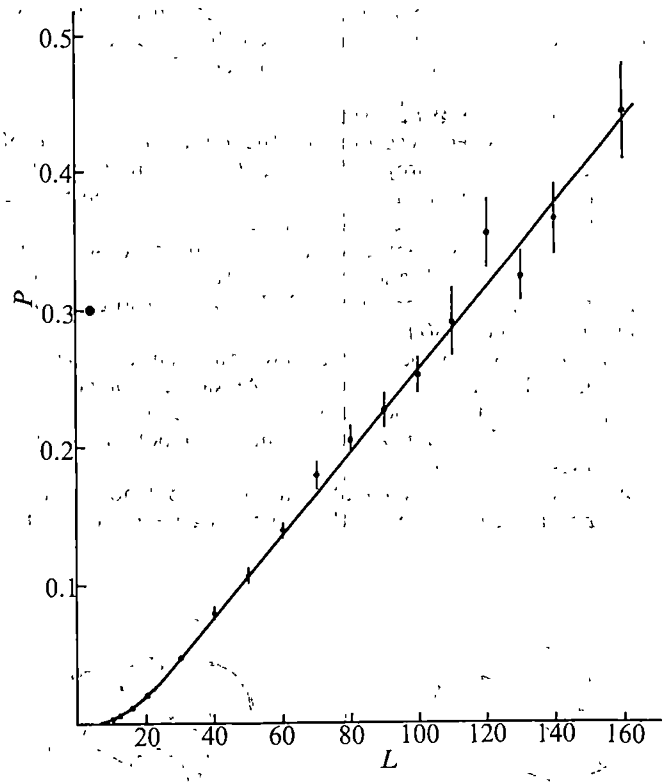

Fig. 4 Probability of non-trivial knot formation during random closing of thin polymer chain involving L segments.

An unexpected feature of the results of Monte-Carlo calculations of P_0 for four chains length (chain length in each pair assumed to be equal), (Fig. 5) was that all follow equation (1), within the limits of calculation errors

$$P_0 = 1-A_0\exp(-\alpha_0R^3) \qquad (1)$$

where A_0 and α_0 are functions of chain length L (Table 3). But the dependence of P_0 on R is the same inside the entire region of lengths studied. The dependence of the free energy of the system, which can be treated as the 'potential' for interaction between chains, on interchain distance is given by the following equation

$$F_0 = -kT\ln P_0 \qquad (2)$$

In summary, the topological restrictions arising in the system of two closed unlinked polymer chains lead to a repulsive potential of interaction between them, having an entropic origin (equations (1) and (2)).

Such interaction between chains must lead to the non-ideal behaviour of a gas or solution containing closed polymer molecules. In the case of the equation for the state of such a

Table 2 Fraction (%) of knots of different types formed during a random closing of a thin chain

Chain length L	3_1	4_1	5_1	5_2	All others
10	98	2	0	0	0
20	83	10	1	3	3
40	76	12	5	3	4
60	66	13	4	6	11
80	58	13	6	6	17
100	58	14	3	6	19
120	58	9	2	6	25
140	53	8	2	9	28
160	46	10	5	8	31

gas (or solution), the second term of virial expansion must arise

$$p = ckT(1+cB), \qquad (3)$$

where c is the number of polymer molecules in the unit volume of gas (or solution), and B is the second virial coefficient. This value is determined by the interaction between a pair of molecules and may be calculated from the potential for interaction between them (for example, ref. 24). In our case, for the potential determined by equations (1) and (2) the calculations lead to

$$B = (2/3)\pi(A_0/\alpha_0). \qquad (4)$$

Values of B calculated from equation (4) for four values of L are given in Table 3. How large has the effect of mutual repulsion of closed polymer chains originating from topological interaction between them proved to be? To answer this question we have compared values of B with those for a hypothetical gas of rigid impenetrable spheres having dimensions corresponding to the mean-square dimensions of polymer coils under consideration. A mean-square radius of gyration of the closed polymer chain was chosen as the radius of a corresponding rigid sphere[2]. Values of B^* for such a gas of equivalent rigid spheres are given in Table 3. At small L, B is smaller than B^* but these values become very close as chain length increases, indicating that the effect of topological interaction between closed unlinked polymer chains effectively forbids any mutual penetration of the chains. Note that this effect originates only from topological restrictions and takes place at the Flory theta point in the absence of any common excluded-volume effects.

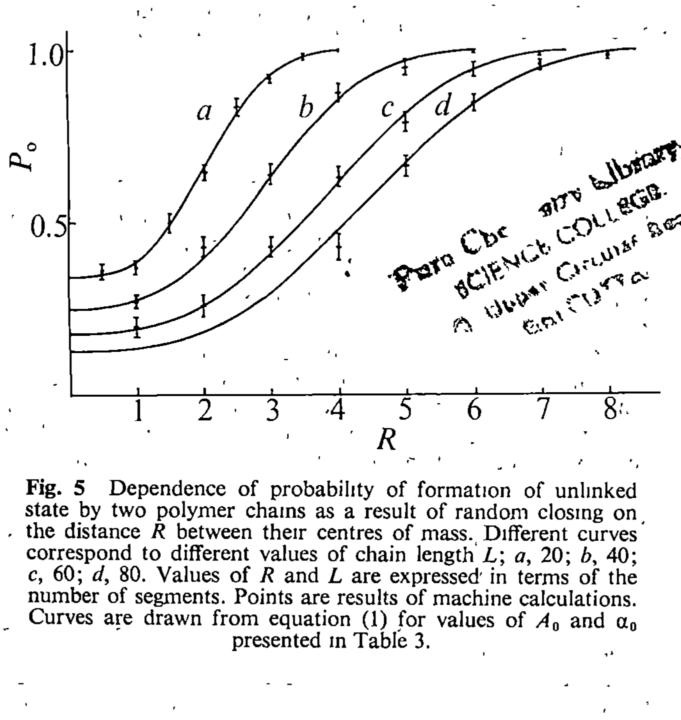

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Fig. 5 Dependence of probability of formation of unlinked state by two polymer chains as a result of random closing on the distance R between their centres of mass. Different curves correspond to different values of chain length L; a, 20; b, 40; c, 60; d, 80. Values of R and L are expressed in terms of the number of segments. Points are results of machine calculations. Curves are drawn from equation (1) for values of A_0 and α_0 presented in Table 3.

Discussion

Theoretical consideration of statistical-mechanical properties of closed polymer chains encounters great difficulties because the topological state of the chains has to be taken into account. It is possible to overcome such difficulties using results obtained in algebraic topology, on the one hand, and the Monto-Carlo method on the other. As a result the calculations of statistical-mechanical properties of chains involving as many as 150 segments have been carried out.

Our calculations relate to an unsolved problem in the search for a closed molecule which forms a knot[5,9,16]. Our results show that for flexible chains, to which the common hydrocarbon polymers belong, the probability of knot formation during their random closing is extremely low (Table 1). Moreover, this probability increases slowly with chain length and for chains containing as many as a hundred segments it is as small as $\approx 10^{-4}$. The search for knotted molecules obtained as a result of random closing of flexible polymer chains does not show much promise. Much more promising is the method of random closing in the case of rigid chains. Double-helical DNA molecules fall into this category, because their segment length exceeds the width by a factor of 50. Some DNA molecules such as DNA from bacteriophage λ are very promising in the search for knotted molecules, as in linear form they have cohesive ends and may be readily closed *in vitro*[6,7]. Our calculations predict that about 40% of the λ DNA molecules which contain about 150 segments should be knotted after closing *in vitro*. Knotted molecules may be easily confused with unknotted ones during electron microscopic inspection of a preparation; in the search for knotted molecules special precautions are needed. Such a search in preparations of closed λ DNA *in vitro* have not been undertaken.

Knotted DNA cannot finish the replication cycle because two daughter molecules will be linked with each other. Thus, the great probability of knot formation even for such comparatively short molecules as λ DNA predicted by our calculations, indicates that inside the cell a special mechanism must be found which forbids the formation of a knotted chain during DNA closing *in vivo*. To rule out this possibility completely requires the location of the DNA molecule on a two-dimensional surface. The cell membrane is best suited to this purpose. Data have lead to the hypothesis about the location of λ DNA inside the cell on a membrane (see ref. 6, p. 100). Naturally, other more specific mechanisms forbidding knot formation inside the cell may be assumed.

The topological interaction between two closed polymer chains may considerably affect the dimensions of a DNA molecule inside the helix–coil transition interval because melted regions represent such closed polymer chains. Such closed chains must be practically impenetrable, one for another, thus simulating large excluded-volume effects. Such anomalous excluded-volume effects have been observed for partially melted DNA by Shugalii *et al.*[12]. They have shown that dependence of DNA specific viscosity on temperature may be understood only if a complete absence of mutual penetration of coiled regions is assumed. They[12] have also postulated that topological restrictions play an important role in this effect. Our results make such interpretation probable. Note, however, that in real experimental conditions a contribution from the common excluded-volume effect as well as from kinetic effects may be considerable. Thus, strictly speaking, the purely topological effects may be demonstrated only if the lack of mutual penetration of closed chains and free penetration of linear chains would occur in the same conditions. In addition to a quantitative estimation of the value of topological interaction our results enable an estimation of the probability of formation of linked molecules (catenanes) during random

Table 3 Parameters of the potential for interaction between unlinked closed polymer chains (α_0, A_0) and values of B for different values of chain length, L

L	α_0	A_0	B	B^*
20	8.2×10^{-2}	0.66	17	36
40	2.6×10^{-2}	0.75	60	101
60	1.2×10^{-2}	0.82	143	185
80	0.8×10^{-2}	0.87	228	286

closing of polymer chains in solution. The concentration of catenanes c_k may be calculated from:

$$c_k = c^2B \qquad (5)$$

where c is the concentration of closing molecules. Note that equation (4) and Table 3 give the value of B expressed in units of l^3, where l is segment length. Thus, to obtain an absolute value of B the value obtained using equation (4) and listed in Table 3 should be multiplied by l^3. In addition, equation (5) is derived using the assumption that the chains have not interacted before closing and that $cB \ll 1$. Our results lead to conclusions about the probability of catenane formation, which are in accord with previous predictions[9]. We know of only one attempt to obtain data on the probability of catenane formation[25].

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Heat resistance of bacterial endospores and concept of an expanded osmoregulatory cortex

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The extreme resistance of bacterial endospores to heat may result from dehydration of the central protoplast brought about and maintained by osmotic activity of expanded electronegative peptidoglycan polymer, and positively charged counterions associated with it, in the surrounding cortex. The cortex may thus act as a specialised osmoregulatory organelle. Changes in the environment which would be expected reversibly to affect osmotic properties alter the heat resistance of spores.

ALTHOUGH many of the enzymes and other macromolecular components of bacterial endospores are as heat-sensitive as those of vegetative forms when extracted from disrupted cells, the resistance of the intact spores exceeds that of the corresponding vegetative forms by factors as great as 10^5-fold[1]. The calcium dipicolinate complex has been widely quoted[1] as being important in heat resistance, but considerable doubts have been raised by the isolation of mutants which produce dipicolinic acid-free spores and which are nevertheless still heat resistant[2]. Lewis *et al.*[3] suggested that such extreme resistance might result partly from dehydration of the central protoplast within the spore by compressive contraction of the surrounding cortex. We[1] reasoned that based on the known properties of peptidoglycan, of which the cortex is largely composed[4], its mass, and the volume occupied by this structure in spores, the cortex was more likely to be expanded than contracted, a possibility pointed out by Alderton and Snell[5]. Peptidoglycan in spores is electronegative and loosely cross linked[6,7] and could therefore be expanded by electrostatic repulsion of adjacent negatively charged groups in the polymer. Expansion would be expected in the absence of high concentrations of cations, while contraction or collapse would be expected in the presence of high concentrations of cations, and particularly in the presence of multivalent ones. Such cation-induced contraction of isolated vegetative cell wall peptidoglycan has been described[8,9].

We[10] therefore tested the expanded cortex hypothesis by treating spores in ways that would be expected to cause collapse of the expanded structure, and then measuring the heat resistance of the treated spores. The predicted heat sensitising occurred in the presence of high concentrations of multivalent cations as long as the spores had been treated in some way to increase permeability of the outer spore coat, for example, by the use of coat-defective mutants, or by chemical modification of the coat, with combinations of urea with thiol compounds, or by the use of a divalent cation ionophore to increase the cation-permeability of the spore.

Table 1 Heat sensitivity of variously treated spores of *Bacillus cereus* in calcium chloride

Treatment*	Survivors (%) after heating† in Water	Survivors (%) after heating† in Calcium chloride
Untreated control	85	98
Urea	105	83
Mercaptoethanol	105	88
Urea+ethanol	83	93
Urea+mercaptoethanol	83	0.23
Urea+thioglycollic acid	78	0.35
Urea+dithiothreitol	101	5.2

B. cereus T spores were grown on potato glucose yeast extract agar at 30 °C and washed and cleaned as described in ref. 10.

*Spores (about 10^8 per ml) were incubated with reagents at 37 °C for 1 h then washed by centrifuging in water. Reagent concentrations were: urea, 7.2 M; β-mercaptoethanol, 10% (v/v); thioglycollic acid, 1% (v/v); dithiothreitol, 6.5% (v/v); ethanol, 10% (v/v). Reagents used at pH 3 and at pH 7 gave essentially similar results. † Treated, washed spores were heated in water or $CaCl_2$ solution (4 M) at 70 °C for 30 min.

This article reviews the evidence and presents new data that support the expanded cortex hypothesis and which also indicate a new possibility, that the cortex functions to maintain the enclosed spore protoplast in a relatively dehydrated state not solely by expansive pressure, but also by osmosis. Osmoregulation by the cortex can be influenced experimentally with predictable effects on the heat resistance of the spore

Heat sensitisation of spores with cations

Tables 1 and 2 summarise data showing that: (1) mutant spores and spores treated with thiol compounds in urea, so that the coats become permeable even to molecules as large as lysozyme[11–13], became heat sensitive in the presence of high concentrations of salts of multivalent cations; (2) in contrast, normal untreated spores of various species, and spores treated with various control reagents that did not contain thiol compounds, remained heat resistant in high concentrations of calcium chloride; (3) effectiveness of the multivalent cations increased in the order Sr^{2+}, Ca^{2+}, Mg^{2+}, La^{3+}; (4) low concentrations of multivalent cations (that is less than molar $CaCl_2$) were relatively ineffective; (5) monovalent cations (for example, Na^+, K^+) did not cause heat sensitisation at high concentrations, equiosmolar with the multivalent cations; (6) thiocyanate and guanidinium ions, which are among the most effective ions known to decrease the stability of proteins to heat[14], did not decrease the heat resistance of the treated spores. The extent of heat sensitisation by multivalent cations was great; that is generally well over 1,000-fold after heating for 10 min at 70 °C, although survivor curves were nonlinear (Fig. 1). In separate experiments the heat sensitivity of urea–mercaptoethanol-treated spores in calcium chloride was reversed if the salt was removed, for example, by centrifuging and resuspending the spores in water before heating[10].

That the heat-inactivated spores were truly non-viable, rather than simply made dormant through inactivation of their germination mechanism, was suggested by the failure of techniques such as washing the spores in chelating agents (sodium dipicolinate, sodium ethylene diamine tetraacetate) and incubation in calcium dipicolinate agar[15] or agar containing lysozyme[16] to induce their recovery (Table 3); such techniques often recover viable but heat-damaged spores. The observations summarised in Tables 1 and 2 are compatible with the hypothesis that the heat resistance of spores is normally maintained when the cortex peptidoglycan is electrostatically expanded and that heat resistance is reduced when the peptidoglycan is electrostatically cross linked and collapsed; for example, sufficient concentration of multivalent cations is allowed to penetrate the permeable coats of mutant or thiol-treated spores. There is considerable evidence that the central protoplast in spores is relatively dehydrated[17]. The expanded cortex hypothesis supposes that expansive pressure in the cortex brings about this dehydration mechanically. Lewis *et al.*[3] pointed out that a pressure of about 50 atm would be sufficient, for example, to compressively dehydrate wool keratin to 15% water.

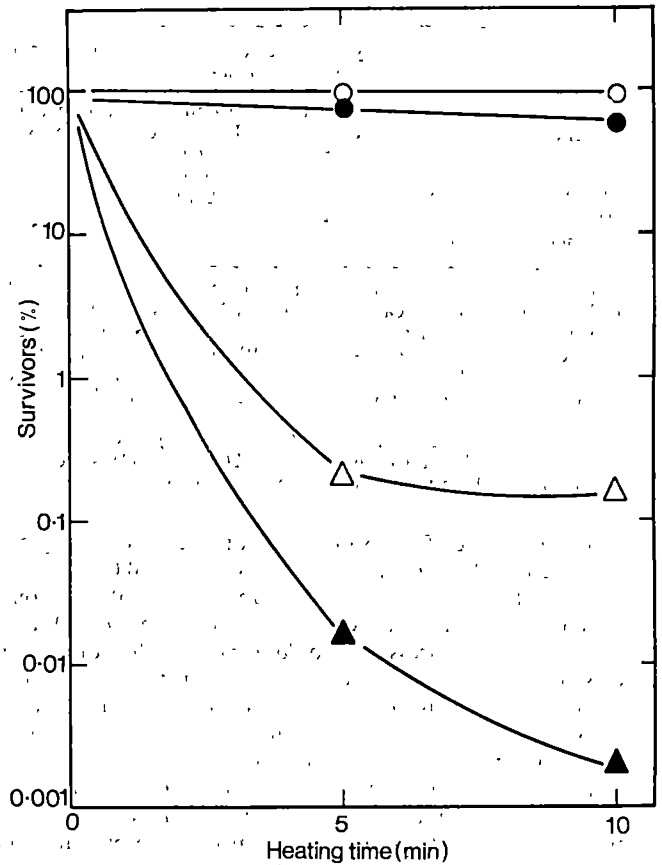

Fig. 1 Heat sensitivity of spores of *Bacillus megaterium* NCTC 7581 in $CaCl_2$. Spores were treated with urea+mercaptoethanol as indicated in Table 2 then washed and heated at 70 °C in $CaCl_2$ solutions at the concentrations indicated in the figure. ○, water; ●, 2 M $CaCl_2$; △, 3 M $CaCl_2$; ▲, 4 M $CaCl_2$.

Alternatively, the cortex could bring about dehydration of the enclosed protoplast more plausibly by other means. Since it contains a loosely cross linked polymer with an excess of free carboxyl groups over amino groups[6,7], the cortex will normally contain a sufficient concentration of free positively charged counterions to maintain electrical neutrality: the polymer plus counterions together will be

Table 2 Effect of salts on heat sensitivity of coat-modified spores

Organism*	Treatment†	Survivors (%) after heating‡ in Water	NaCl (4 M)	KCl (4 M)	$SrCl_2$ (4 M)	$CaCl_2$ 4 M	$CaCl_2$ 3 M	$CaCl_2$ 2 M	$CaCl_2$ 1 M	$MgCl_2$ (4 M)	$La(NO_3)_3$ (4 M)	KNCS (5 M)
B. cereus T	Untreated control	85	89			98						100
B. cereus T	Urea+mercaptoethanol	100	90	64	1.1	0.85	7.8	58	77	0.1	0.01	100
B strearothermophilus	Urea+mercaptoethanol	90				0.47	1.6	24	49			
B. megaterium NCTC 7581	Urea+mercaptoethanol	100				0.0012	0.08	30	55			
Cl. sporogenes NCIB 2111	Urea+mercaptoethanol	100				1.8						
Cl perfringens 8 6 (coat-defective[12])	Untreated	98	97			0.1		2.9				
B. megaterium ATCC 9885 (coat-defective[13])	Untreated	100				15			90			

*Spores were produced and cleaned as in ref. 10.

†Conditions of treatment as in Table 1 except incubation was at 60 °C for 1 h.

‡Heating conditions as in Table 1 except for *Cl perfringens* (100 °C, 10 min) and *B. stearothermophilus* (100 °C, 30 min).

NaCl and $CaCl_2$ solutions adjusted to *p*H 3 and *p*H 7 with HCl or NaOH gave similar results to those shown

Table 3 Attempted recovery of spores of *B. cereus* T heat-inactivated in calcium chloride

Treatment*	Survivors (%)† enumerated in media Control agar	Lysozyme agar	CaDPA agar
Control	0.43	0.56	0.54
NaEDTA	0.38	0.40	0.42
NaDPA	0.44	0.48	0.54
Urea+mercaptoethanol		1.06	

*Spores were first treated with urea+mercaptoethanol (60 °C, 30 min) then inactivated by heating in $CaCl_2$ (4 M, 70 °C, 30 min). Survivors were either plated directly (control) or washed with sodium ethylene diamine tetraacetate (NaEDTA, 100 mM, pH 7), or sodium dipicolinate (NaDPA, 100 mM, pH 7) or reincubated with urea+mercaptoethanol (60 °C, 30 min) to ensure maximal lysozyme sensitivity before plating

†Medium was plate count agar (PCA, Oxoid). Lysozyme was incorporated in PCA at 100 μg ml^{-1} and 20 mM CaDPA (ref. 15), a concentration which is germinative for this organism

osmotically active. For example, assuming a 30% polymer gel of high molecular weight (for example, 20,000) in the cortex, with three carboxyl groups per 1,000 daltons, the osmotic pressure exerted could be in excess of 30 atm Such an osmotic pressure would certainly dehydrate the enclosed protoplast provided that the protoplast did not contain high levels of low molecular weight soluble components, since it is the molarity that largely determines the osmotic pressure. (In fact, the major low molecular weight components in spores are few—principally dipicolinic acid, glutamic acid and other acidic components[18] which, with the high levels of calcium that are present, will be mostly insoluble at physiological pH values) For example, a solution of the single pure protein haemoglobin is at equilibrium with a solution at 30 atm at a water content of only about 30% (ref. 19) If proteins and other components in the spore protoplast are condensed or associated into multimeric units, their osmotic coefficients will be reduced[20] and the water content could well be even below this and closer to the values of 20% or less that have been indicated by refractive index measurements of spores[21,22]

Osmotic manipulation of spore heat resistance

If the heat resistance of bacterial spores depends on osmotic dehydration of the protoplast by the surrounding cortex, one can predict that heat resistance should respond to osmotic manipulation. Therefore, to test the hypothesis further, spores of *Bacillus cereus* were first treated with urea plus mercaptoethanol, as in Table 1. These spores were then heated in calcium chloride plus sucrose at a concentration estimated to give an osmotic pressure high enough to maintain dehydration of the spore protoplast Spores heated in this mixed solution were found to have retained resistance to heat (Table 4). Thus, the observed heat sensitisation in calcium chloride is explicable in terms of electrostatic cross linking of peptidoglycan electronegative groups, loss of counterions causing a decrease in osmotic pressure in the cortex, and consequent rehydration of the enclosed protoplast· re-establishment of heat resistance in sucrose is explicable in terms of dehydration of the rehydrated protoplast, brought about simply by osmosis. A problem remaining is that the calcium chloride itself will exert an osmotic pressure even greater than that exerted by the sucrose and should also cause dehydration, unless it is able to permeate the protoplast.

The level of sucrose needed to maintain the resistance of such sensitised spores of *B. cereus* was found in separate experiments to be above 2 M. Sucrose at 2 M would exert an osmotic pressure of more than 40 atm and one might infer that this value represented a lower limit for the osmotic pressure normally existing within the cortex of spores of this organism. That such protection occurs is not remarkable since protection of vegetative cells from heat inactivation by dehydration (and plasmolysis) by solutions of non-permeant solutes like sucrose is well known, and the magnitude of the protection can reach several hundredfold[23].

Mechanism of spore heat resistance

Figure 2 summarises the new hypothesis, which has a number of attractive features It is compatible with the observed high total water content of spores (about 70%) as measured by Black and Gerhardt[24] and yet allows for a relatively dehydrated protoplast (in accordance with the high refractive indices of spores[21,22]), since the cortex region of the spore would be relatively water-filled although in osmotic equilibrium with an enclosed, water-depleted protoplast. Presumably, since water does not continually diffuse into the spore, the system comes into equilibrium as a result of mechanical constraints and pressures developed by the cortex. Osmoregulatory and pressure-imposing functions of the expanded cortex are therefore interdependent. The high apparent specific gravity of spores in equilibrium gradient centrifugation experiments (that is sometimes > 1.4, ref. 25) is readily explained since the low molecular weight solutes used to form such gradients would be expected to permeate the cortex space[26], and therefore indicate dry spore rather than wet spore density The concept of an osmotically active cortex accommodates well the observations that spores held at low equilibrium relative humidities (ERH) show greater water sorption than vegetative cells[27,28]. The concept is compatible with the increase in resistance that occurs when spores are suspended in certain highly concentrated solutions[29], and also with the observations that the resistance, in particular, of relatively heat-sensitive spores, increases greatly with decrease in ERH[30], for equilibration at a sufficiently low ERH would lead to loss of water from the cortex, causing an increase in osmotic pressure and consequent further dehydration of the enclosed protoplast as equilibrium was re-established. The hypothesis does not conflict with the observations of Alderton and Snell[31] that treatment with acid reduces the heat resistance of spores, since such treatment would displace positively charged counterions from the cortex by protonating peptidoglycan carboxyl groups, thus reducing expansion and lowering osmotic pressure. Re-equilibration at high pH values would be expected to reimpose expansion, increase the osmotic pressure exerted by the cortex and reinstate heat resistance, as is found experimentally[31].

The hypothesis predicts that the outer regions of spores (cortex and coats), being relatively wet, should collapse on drying Surface collapse was inferred by gas displacement studies[32] and by light scattering analyses of single spores[33]. That the cortex is normally expansive is also indicated by electron micrographs of disrupted spores in which cortex material seems to expand into the space vacated by the extruded protoplast contents[34].

If the cortex is an electronegative expanded polymer, then an expansion mechanism must operate during sporulation. Sporulation specific peptidases have been described that hydrolyse peptidoglycan peptide components[35] and may operate to allow controlled expansion. Alternatively,

Table 4 Osmotic reversal of the heat-sensitisation of *B. cereus* T spores

Heating menstruum	Survivors (%) following heating*
Water (control)	100
Sucrose	123
$CaCl_2$	0.69
$CaCl_2$+sucrose	84.2

*Spores were incubated with urea+mercaptoethanol as indicated in Table 2 then heated at 70 °C for 30 min in water or in $CaCl_2$ (3 M) containing no further addition or containing sucrose (3.6 M).

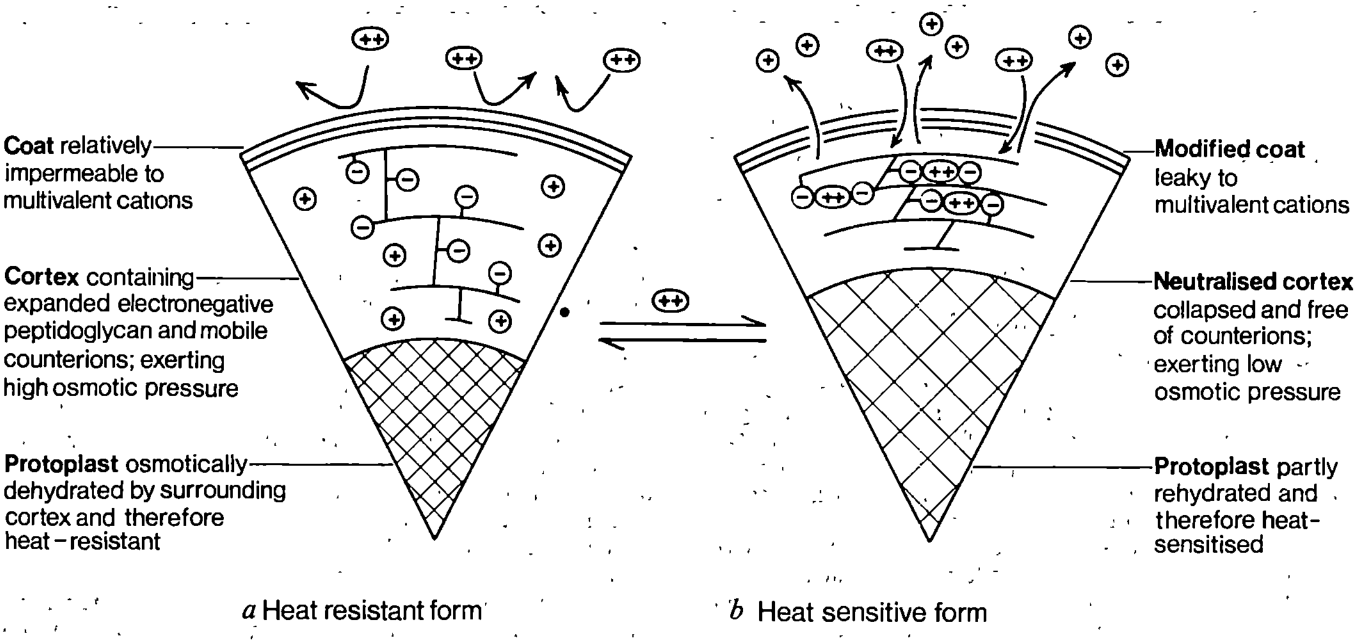
++
++
++
Coat relatively impermeable to multivalent cations
Cortex containing expanded electronegative peptidoglycan and mobile counterions; exerting high osmotic pressure
Protoplast osmotically dehydrated by surrounding cortex and therefore heat-resistant
++
+
++
++
Modified coat leaky to multivalent cations
Neutralised cortex collapsed and free of counterions; exerting low osmotic pressure
Protoplast partly rehydrated and therefore heat-sensitised
a Heat resistant form
b Heat sensitive form

Fig. 2 Diagrammatic representation of the osmoregulatory expanded cortex in normal heat-resistant spores (*a*) and in coat-modified spores made heat sensitive by multivalent cations (*b*). —, Negatively charged groups on cortex peptidoglycan, +, monovalent positively charged mobile counterions, ++, multivalent cations.

removal of cross linking cations from this region (for example, by newly synthesised dipicolinic acid) could bring about expansion and the consequent increase in osmotic activity. In its final form, spore peptidoglycan is certainly more electronegative and less cross linked (that is, more expandable) than vegetative polymer[6], and the relative content of the major electronegative component, diaminopimelic acid, has been shown to be particularly high in spores of the most heat-resistant species[36].

The fact that only spores with modified coats are susceptible to heat sensitisation by multivalent cations suggests that the coats of normal spores have an important cation-protective role. This is open to further study.

The hypothesis has several important implications regarding the dormancy of spores. Calcium dipicolinate, which is present within bacterial spores, will initiate germination if added exogenously[37]. It is possible that endogenous calcium, which is present at high levels in spores, performs this task during normal germination. Movement of sufficient calcium into the cortex compartment could neutralise electronegative groups, leading to cortex collapse and replacement of counterions. Dring and Gould[38] found release of spore-bound K^+ to be the earliest event in germination of spores of *B. subtilis*, occurring at the time when dormancy and heat resistance are rapidly lost[39], and it is interesting to speculate that this K^+ might represent counterions displaced from the cortex compartment. The extreme dormancy of dipicolinic acid-negative, low calcium mutant spores[2] may reflect their inability to neutralise the cortex polymer.

Finally, the idea that osmoregulation within a single cell by an organelle like the cortex contributes to resistance, and possibly also to dormancy, may not be peculiar to bacterial endospores. Maintenance of dehydration is typical of resistant and dormant systems in diverse prokaryotes and eukaryotes, and thick-walled structures around resting bodies such as spores, conidia and cysts are common[40]. Some of these contain electronegative polymers, for example, polyuronic acids in *Azotobacter* cysts[41]; peptidoglycans in microcysts of *Myxococcus xanthus*[42] and spores of *Streptomyces*[43]. Examination of the possible osmoregulatory functions of such structures may prove worthwhile.

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letters to nature

A very high velocity 1,612-MHz OH source near the galactic centre

Using the 25-m radiotelescope at Dwingeloo, we have recently completed the initial stages of a large scale OH survey. The detailed results will be reported later. The survey is designed to yield an unbiased set of data for all four 18-cm lines of OH.

The first part of the survey project was carried out at a frequency of 1,612 MHz with a closed-cycle helium-cooled parametric amplifier and a system noise temperature of 42 K on cold sky. We used a 256-channel autocorrelation spectrometer in a frequency switched mode with a total bandwidth of 2.5 MHz (a total velocity coverage of 465 km s^{-1}) switching over 2.5 MHz. At 18 cm the HPBW of the Dwingeloo telescope is 31′.

A region of about 12 square degrees around the galactic centre was among the areas observed. It was sampled every 0.3° in both galactic longitude and latitude. Here we discovered a double-peaked absorption feature close to the galactic centre which appeared in several adjacent grid points. Subsequent analysis, however, showed it to be an emission feature in the reference band and therefore at a high velocity.

Confirmatory observations were obtained on August 7, 1975 with the dual-channel 18-cm receiver (system noise temperature of 120 K on the galactic centre) of the 100-m telescope in Effelsberg. The instrument was used in a total power mode with the two halves of the 384-channel autocorrelation spectrometer arranged to receive the two orthogonal polarisations, which were then averaged. Figure 1 shows the final spectrum measured in this manner. The form of the line profile and the velocity separation of the two components (26 km s^{-1}) is typical of that of type IIb OH/IR stars[1]. The integrated powers of the two components are approximately equal, that of the lower velocity component being 8.0×10^{-22} W m^{-2} and that in the higher velocity component being 7.8×10^{-22} W m^{-2}. Both components seem to consist of at least two features with our frequency resolution of 6.5 kHz.

The most striking characteristic of this source, however, is its high mean radial velocity of −341 km s^{-1}. From interpolation across a grid of observed points we derive its position to be

$$l^{II} = 0.330\pm0.005°, \quad \mathrm{RA}(1950) = 17\,\mathrm{h}\,43\,\mathrm{min}\,59.0\pm1.2\,\mathrm{s}$$
$$b^{II} = -0.193\pm0.005°, \quad \mathrm{dec}\,(1950) = -28°\,44'\,13\pm18''$$

The source seems to be pointlike with the 7.9′ beam of the 100-m telescope. Its projected distance from the galactic centre is 67 pc (assuming the Sun is at a distance of 10 kpc). A search of this confused region on the Palomar Sky Survey reveals no unambiguous optical counterpart.

High velocities are not uncommon in the direction of the galactic centre[2]. Rougoor and Oort[3] have given a value of ±200 km s^{-1} for the radial velocity range for interstellar material physically close to the centre. The velocity of the source we have found, however, is far outside this range. Comparison with Thackeray's list[4] of optical high velocity objects shows that the observed velocity is among the highest found in the Galaxy. This may be considered as supporting evidence that the source has a stellar nature and is unlikely to be of population I (refs 5, 6).

A further striking characteristic is that, given the velocity and galactic longitude, this object seems to be moving counter to the general galactic rotation. This property is shared with further sources, at more modest velocities, found during the above survey.

It is a tantalising possibility that many more such sources exist near the galactic centre. This may be studied with a deep, high velocity survey. If a sufficiently large number of similar sources are found, one can then derive their space and velocity distributions and, as a consequence, calculate the gravitational potential and mass distribution close to the galactic centre. Optical studies by Minkowski[7] on the radial velocity distribution of planetary nebulae near the centre were hampered by the interstellar extinction in that direction. Such a deep high velocity survey has the advantage of studying a well-relaxed subsystem of the central galactic bulge (no worry about non-circular motions[3]) by radioastronomical observations (no interstellar extinction[8]). Further observations are in progress.

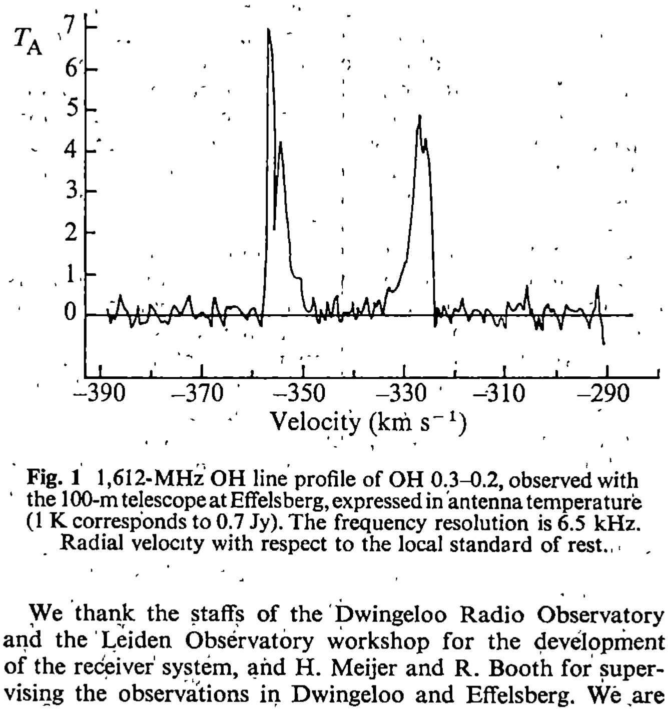

Fig. 1 1,612-MHz OH line profile of OH 0.3–0.2, observed with the 100-m telescope at Effelsberg, expressed in antenna temperature (1 K corresponds to 0.7 Jy). The frequency resolution is 6.5 kHz. Radial velocity with respect to the local standard of rest.

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Late type giants in Large Magellanic Cloud

WITH objective prisms used on a Schmidt telescope, faint late type stars may be identified with a dispersion of 1,700 Å mm^{-1}, at the atmospheric A band, on photographic plates sensitive in the near infrared spectral region. The required spectral classification techniques were developed by J. J. Nassau and various collaborators[1–3]. These techniques were used by Westerlund[4,5] with the Mt Stromolo-Uppsala 50/66-cm Schmidt telescope to survey the M-type supergiants in the Large Magellanic Cloud (LMC). Westerlund found relatively large numbers of early type M stars brighter than $m_{\rm I}=13.0$ in the photometric system of Kron, Gascoigne, and White[6].

With a new objective prism[7] which yields 6,700 Å mm^{-1} at the A band attached to the 61/91-cm Curtis Schmidt telescope, late type M stars may be classified to approximately $m_{\rm I}=16.5$. This prism makes possible the detection and temperature classification of M type stars later than M1, but late M stars are easier to recognise than the earlier types. The absolute visual magnitude of luminosity class III late M giants in the Solar neighbourhood is approximately -0.9 and the $(M_{\rm V}-M_{\rm I})$ colour of an M6 giant is about 3.5. Thus in the LMC whose apparent distance modulus is ~ 18.6, it is possible to segregate and to classify these stars since they should show up at about $m_{\rm I}=14$ if they are physically similar to their galactic counterparts. Since this magnitude is appreciably higher than the limiting magnitude ($m_{\rm I}=16.5$), it seemed worthwhile to carry out a search for such stars in the LMC. We present here preliminary results of such a search.

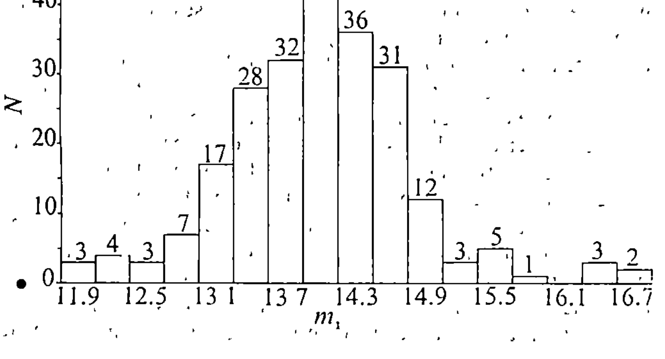

Fig. 1 The 233 stars of types M6 and later plotted according to their observed apparent infrared magnitude, $m_{\rm I}$. Magnitudes were interpolated from photoelectric measures on the BV system[11] and converted to the infrared scale[15]. The limiting mangitude for the survey is $\sim m_{\rm I}=16.5$. The stars are grouped in magnitude intervals of 0.3 $m_{\rm I}$; the range of magnitudes measured extends from $m_{\rm I}=11.75$ to $m_{\rm I}=16.85$ Maximum frequency occurs at $m_{\rm I}=14.0$, which being well above the plate limit, seems to be a real effect A group of 225 stars of types M6 and later in the interval $m_{\rm I}=12.5$ to $m_{\rm I}=15.8$ were used to compute the dispersion about the observed mean apparent magnitude at $m_{\rm I}=14.0$; the value of this dispersion is ±0.6 mag.

Two Kodak I N plates, hypersensitised in ammonia solutions, were used for the spectroscopic survey in the LMC. For photometric estimates two direct non-sensitised plates were used. To minimise the effects of possible variability among late M-type stars, all the observational material was gathered within an observing period of one week during October 1974. Interstellar reddening at the observed wavelengths is small, and in this preliminary survey has been neglected.

Stars of class M 6 5 and later seem to form a natural group according to the definitions of Morgan[8], even when observed with the small dispersions given by the new objective prism. The characteristic feature of this natural group is an apparent drop in the intensity of the continuum at wavelengths longer than 8,400 Å. This is caused by a blend[9,10] of TiO 8,432 Å and 8,570 Å bands and the VO bands at 8,575 Å and 8,624 Å. The present study was limited to stars that belong to this group. The spectral classification technique used here yields only temperature classifications. In the absence of luminosity criteria one must rely on statistical arguments[11,12], which show that the overwhelming majority of stars segregated in a survey such as this are giant stars. The distributions found here according to position and to apparent magnitude leave no doubt that this is the case.

An area of 8.75 square degrees centred at RA 5 h 24 min and dec. $-70.0°$ (1975) was surveyed. The centre of this area is close to the optical centre of the LMC and to its centre of gravity as found from the rotation of the Galaxy determined from 21-cm observations[13].

The number of late M stars found is 803. Their surface distribution suggests a symmetrical concentration about the centre of the area where 120 late M stars per square degree are found. This surface density decreases by about a factor of 2 at a distance of 1.25° from the centre, except in the direction of the Bar, where a 20% excess of stars is found.

Apparent ($m_{\rm I}$) magnitudes were obtained for 228 of the late M-type stars. Photometric sequences were formed by using 68 OB stars for which Lucke[14] had published B and V magnitudes. The $m_{\rm I}$ magnitudes were derived from a $(B-V)$, $(V-I)$ relationship established by Blanco[15]. The $m_{\rm I}$ distribution shows a maximum of $m_{\rm I}=14.0\pm0.01$. The standard deviation of the $m_{\rm I}$ distribution is ±0.6. This result suggests that the stars found in this survey may be similar to stars of class M6 III and M7 III in the solar neighbourhood. This coincidence is interesting in view of possible differences in the chemical abundances of stars in the Galaxy and in the LMC. It should be pointed out, that evolutionary models relating spectral classes and luminosities for late type M stars of luminosity class III are not available. Furthermore, the spectroscopic criteria employed in a survey such as the one described here may very likely contain a selection effect in which stars with strong TiO and VO features are favoured. All that one may conclude from the results presented here is that both the Galaxy and the LMC seem to show a stellar population component of late type giant stars of luminosity class III, and that these stars may be physically similar in the two galaxies These results suggest that the LMC may have M giant stars contributing to the disk population.

This is a very preliminary report of our survey. Since it is possible that in the use of a technique as new as the one used here unexpected errors may exist, a definitive study will be made in which a large telescope aperture and a grating-prism combination as described by Bowen and Vaughan[16], will be used.

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Transient X-ray sources

IN the eccentric-orbit binary model[1,2], a compact object is in an eccentric orbit around a more massive, slightly evolved star. If the compact object accretes mass from the stellar wind of the normal star, the accretion rate is time dependent, so that the resulting X-ray emission would show a maximum at periastron (in the absence of absorption extinction) and a minimum at apastron. It is also possible that accretion might arise from direct mass transfer resulting from contraction of the Roche lobe with decreasing primary to secondary distance. We show here that the stellar wind model, is adequate to describe the majority of transient X-ray sources.

Eccentric-orbit binaries may be produced when there is a supernova explosion in a close binary system. Usually such an explosion disrupts the binary system, but Gott[3] has suggested that in a significant fraction of such supernovae the collapsed residue of the explosion (a white dwarf, neutron star, or black hole) may be retained in orbit around the runaway second star, the resultant system being a binary with high eccentricity and large space velocity reflecting its former large orbital velocity.

X-ray transients are characterised by a rapid rise, over a few days, to maximum emission, which may last for up to 4 months and then smoothly and progressively decreases. The decrease is approximately that expected by reduced accretion with diminishing stellar wind intensity as the primary–compact object distance increases from periastron. We suggest the following possible explanation for the rapid onset The stellar wind from the primary is slowed by interaction with the magnetosphere of the compact object, and can only enter this through the 'cusps' above each magnetic pole, or through the magnetospheric tail. The X-ray emission, which then originates in the hot turbulent gas columns above the magnetic poles, depends on both the temperature and the square of the gas density. It could be that prolonged exposure to the stellar wind is required as the compact object approaches periastron before these factors reach the critical values for appreciable X-ray emission, resulting in an asymmetric light curve. Meanwhile the atmosphere of the primary will show tidal distortion, and this is likely to increase the time after periastron in which accretion can take place, so that the asymmetry of the light curve would be further enhanced. The asymmetry was ascribed by Pacini and Shapiro[2] to varying absorption before and after periastron. The spectral observations of the transient A1118−61 by Ives *et al.*[4], however, show that there is no significant change in column density, so that such an explanation is untenable, as are earlier models for transients which require large changes in the amount of circumstellar material to produce the observed light curves.

Two transients have shown a periodic behaviour with periods of the order of minutes; (A1118—61, 6.75 min; A0535+26, 104 s). Pacini and Shapiro[2] ascribed the periodicity to the intrinsic rotation of the compact star, which, because of the hard X-ray spectrum, is probably a neutron star (although the period of the order of minutes is rather more typical of a white dwarf). Additionally Brecher[5] suggests that the modulation may be related to precession of the neutron star dipole magnetic field axis.

Most of the transient X-ray sources are observed to have 'hard' spectra (characterised by power law indices of ~ 0.8) that are broadly similar to other galactic X-ray sources, in particular the well understood X-ray circular orbit binaries. The eccentric binary model produces X rays by accretion in exactly the same way, thus it is expected that these two classes of objects would have similar spectra. We note, however, that the 'soft' spectrum[6], and the identified optical counterpart of A0620-00 (ref. 7) mark it out as of a distinctive character.

Clark and Caswell[8] have found the characteristic interval between supernovae in the Galaxy to be ~ 150 yr, comparable with recent estimates of pulsar birthrates. Thus it would seem that most supernovae produce pulsars, and an upper limit to the numbers of supernovae in binary systems which give bound runaway systems would seem to be ~ 10%. Thus we would expect 10^3 eccentric-orbit binaries in our Galaxy which are runaways from supernovae which have occurred in the last 10^6 years (the time for orbit circularisation; Lea and Margon[9]). The Ariel V satellite has discovered a large number of strong transient X-ray sources since launch in October 1974. This suggests a lower limit of 6 transients brighter than the Crab X-ray source per year, and ~ 100 transients per year in our Galaxy (see ref. 10). We thus deduce that X-ray transients spend ~ 10% of their time actually on. As transients are observed to last for up to ~ 4 months, this implies orbital periods of a few years for the very eccentric orbit transients. Thus we would expect transients to reappear on time scales of up to a few years.

Two earlier models for X-ray transients may be examined in the light of the recent observations.

Many authors have compared X-ray transients with optical novae because of their similar light curves, but there is so far no convincing evidence for the occurrence of a nova close to an X-ray transient.

Fabian *et al.*[11] have proposed the Mira-type variable RS Cen (lying 4′ outside the Ariel V error box) to be the optical counterpart of A1118−61. Their model predicts regular X-ray outbursts with a period of 164.5 d, the pulsation period of RS Cen, but Copernicus observations of this region exactly two periods before and one period after the peak of A1118−61, showed no evidence for the transient (Davison and Sanford, personal communication). The only remaining possibility is for the orbital period to be exactly three times the pulsation period of RS Cen. Although this is unlikely, it is possible to envisage systems where an expansion of the primary increases the stellar wind and so the accretion rate, giving a bright source. The contracting of the primary would then lead to the decline. This model makes several predictions: first the light curve will be determined by the rate of expansion and contraction, second if the range of the binary period is hours to weeks some indication of this may be expected in the light curve from eclipses, or, if the compact object is rotating this period would show the Doppler effect, and thirdly that the primary would be expected to show some optical brightening. Most of the well observed transients have been found to fit the synthetic light curve of Amnuel *et al.*[12], and it is hard to imagine how stars with very different masses can expand and contract in identical manners. In addition no evidence has yet been found to support the second prediction. The suggested optical identification for A0620−00 provides the only possible evidence for the third prediction, but as already noted, this seems to be a distinctive type of object.

By generalising these arguments to include Roche-lobe overflowers, we envisage a possible evolutionary model for all the compact galactic X-ray sources (the only obvious exception being pulsars emitting by the synchrotron process).

A supernova explosion in a close binary system leaves a collapsed object, which may or may not remain bound to the original secondary. If mass transfer has already taken place, then the supernova occurs in the less massive star, and isotropic mass loss from a symmetric explosion cannot by itself produce an unbound runaway (see, for example, Paczynski[13]). The system could be disrupted by an asymmetric explosion, however, with the production of a runaway collapsed object which, if a neutron star, might be observable as a radio pulsar. Additionally, momentum imparted to the unexploded star by the supernova shell might contribute to the disruption of the system. Tademaru and Harrison[14] have proposed that pulsars may be accelerated by the emission of asymmetric low frequency electromagnetic radiation to attain the characteristic observed velocities, which exceed 100 km s^{-1}.

Binaries that remain bound after the supernova will have an increased period and possibly a high eccentricity related to a high runaway velocity[13,15]. The eccentric-orbit binary model for X-ray transients requires sufficient accretion near periastron (either from the stellar wind or Roche-lobe overflow of the primary) to account for the observed intense X-ray emission. Then after ~ 10^6 yr the orbit will have become circular and synchronised by tidal forces, so that a close (possibly eclipsing) circular orbit X-ray binary may be observed, until the Roche-lobe overflow of the primary extinguishes X-ray emission.

A further supernova in the uncollapsed companion would give, if the system remains bound, a binary with two collapsed components which may be detectable as a binary pulsar, but of the ~ 120 observed radio pulsars only one is seen in a binary system (with two collapsed components[16]). This implies that ~ 1% of binaries remain bound after two supernovae.

We would suggest that the source Cir X-1 is an intermediate period transient. The variability of the source and the difficulty of ascribing an orbital period have been adduced to a highly eccentric orbit with large period[1]. Davison and Tuohy[17] have combined their Copernicus satellite observations of Cir X-1 with earlier observations (W. A. Baity, M. P. Ulmer and L.-E. Peterson, unpublished), and suggest an interval of ~ 220 d between times of peak X-ray emission. The source Aql X-1 has recently shown another 'transient-like' brightening, consistent with it also having an eccentric orbit.

The main observational consequence of the eccentric-orbit model is that all the X-ray transients would be expected to reoccur, the repetition period simply being the binary period of the system, which could be up to several years.

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Enhanced abundances of Ca, Ti and Cr in low energy cosmic rays in Skylab experiment

WE report here the results of the measurements of the abundances of Ca, Ti and Cr nuclei relative to Fe, in low energy cosmic rays in 25–100 MeV/a.m.u. interval, which were measured in a dielectric detector assembly exposed on the outside of Skylab III for 73 d. The collecting power (defined as the product of area and exposure time) of the lexan detector in this experiment was the largest so far used in free space—1.1×10^9 cm^2 s and the period of exposure from November 22, 1973 to February 3, 1974 was exceptionally 'quiet' (free from solar activity). These factors enabled us to make a reliable study of the abundance of these low energy cosmic-ray nuclei. The results on the measurements of the low energy oxygen group of nuclei in the same detector have been reported earlier[1]. We found an interesting feature in the enhanced abundances of low energy Ca–Cr nuclei relative to Fe, which are different from the composition of high energy (> 1 GeV/a.m.u.) cosmic rays, but are similar to those of low energy cosmic-ray nuclei observed in interplanetary space[2,3]. In the present work, however, observations have been made down to an energy of 25 MeV/a.m.u., which is a factor of two lower than those in earlier studies.

The detector consisted of a stack of 32 lexan polycarbonate sheets, each of area 180 cm^2 and thickness 0.025 cm, covered with aluminium foil of a minimum thickness of 27 mg cm^{-2}. This module was exposed on the exterior of Skylab III at an altitude of 435 km in a circular orbit of inclination 50° for 73 d.

Data on heavy nuclei of $Z > 16$ recorded in a part of the detector were analysed. Heavy nuclei of cosmic rays on passing through the detector cause damage to its material, and give tracks visible after chemical etching when viewed through a microscope. The etched cone lengths in consecutive sheets and the positions where the particle has stopped, give information on the charge and energy of the particles. To increase the sensitivity of the lexan sheets, they were irradiated with strong ultraviolet radiation for 12 d (6 d on each side) before etching with 6.25 N KOH solution at 40±0.02 °C for 12 h. About 25 cm^2 of each of the top six sheets of lexan were scanned for tracks of nuclei of $Z > 16$.

The determination of charge was based on the well known cone length (L) against residual range (R) method[4]. The L–R plot for the tracks of $Z > 16$ are shown in Fig. 1, together with some data for $Z = 6$–10 nuclei obtained from scanning a small area of the detector. Our charge calibration is based on the assumption that: (1) the highest charge nuclei of greatest abundance are of iron, and (2) those which are most abundant for $R < 100$ μm are oxygen nuclei. The calibration lines for other charges derived from the L–R values for O and Fe nuclei are shown by dotted lines in Fig. 1. The calculated and observed positions of different charges, as well as the internal consistency of the data, give support to the reliability of the charge assignments. A sample of tracks coming from the bottom side was also analysed and these data also agreed well with our charge assignments. The charge resolution for $Z \geqslant 20$ seen from the Fig. 1 is about $\Delta Z = \pm 1$; it is even better if one takes into account the multiple data for the same track which show good agreement. We have assigned the charge of a track to the nearest even charge, which we believe to be reliable.

The relative abundances derived from the differential fluences $d\varphi/dE$ for time integrated flux are shown in Fig. 2. For a comparison, the relative abundances of galactic cosmic rays of energy > 0.85 GeV/a.m.u. are also shown, normalised to Fe. The most notable observation is the large abundance of Ca, Ti and Cr at low energies relative to Fe compared with high energy cosmic rays.

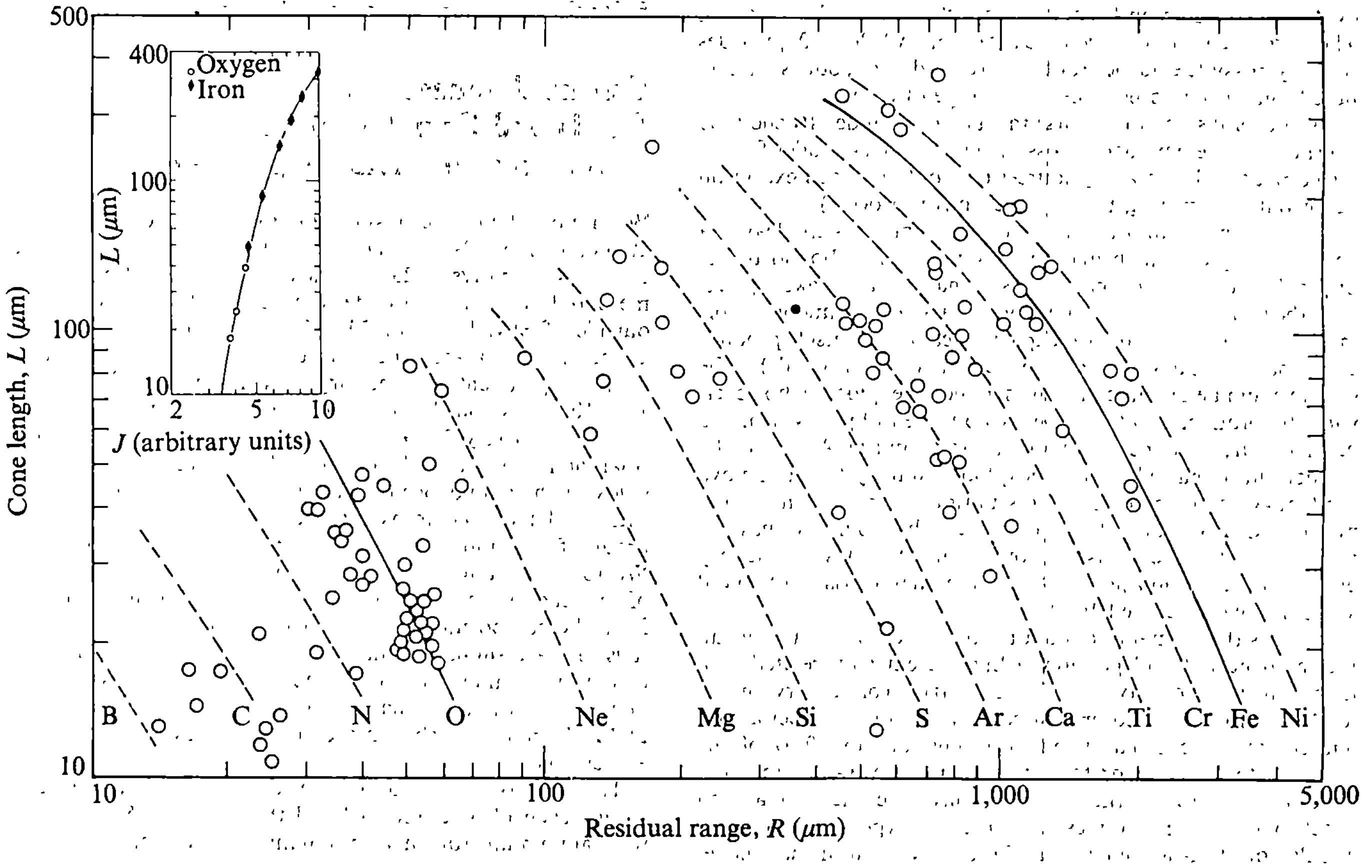


Fig. 1 *L–R* plot for a sample of tracks of C–Ni nuclei in the Skylab detector. The data for C–Ne are from a smaller area scan. The calibrations were carried out by fixing the O and Fe ions (solid lines; see text). The calibration curves for other charges (dotted lines) were derived from the *L–J* (specific ionisation) plot of O and Fe (see inset). The values of *J* were derived using the relation (1) given in ref. 4 with a value of $K = 10$.

Table 1 shows the abundances of Ca–Ni nuclei derived in this work in the energy interval of 25–100 MeV/a.m.u. along with the data measured by other workers[2,3]; at slightly higher energies, on the lunar surface. The abundances in high energy galactic cosmic rays at $E > 850$ MeV/a.m.u. are also shown for comparison[5]. Individual abundances of Ca, Ti and Cr nuclei show high values (relative to Fe) as compared with high energy galactic nuclei. The striking point in our work is the value of 1.5 ± 0.5 for (Ca+Ti+Cr)/(Mn+Fe+Ni) which is a factor of 6.5 ± 2.2 higher than the value of 0.23 ± 0.02 obtained for the high energy galactic cosmic rays. High relative abundances of Ca, Ti and Cr nuclei at low energies are also seen to a lesser extent in the other two interplanetary experiments.

The other significant observation concerns the flux and energy spectrum of these nuclei. In Fig. 3*a*, we have shown the fluences of Fe-group nuclei (Cr–Ni) measured in the Skylab detectors which indicate that the energy spectrum is nearly flat in 25–100 MeV/a.m.u. interval. The interplanetary cosmic-ray flux of Fe-group nuclei has been measured[2,3,6,7], and the differential flux is nearly flat in the region of 20–120 MeV/a.m.u. with an average value of $(4.5 \pm 2.0) \times 10^{-8}$ particles/(cm^2 sr s MeV/a.m.u.). as shown in Fig. 3*b*. We have calculated the expected fluence at the Skylab orbit using a 5% exposure

Table 1 Relative abundances of Ca–Ni nuclei

	Low energy particles			High energy cosmic rays
	Present work	O'Sullivan *et al.*[2]	Fleischer *et al.*[3]	Webber *et al.*[5]
Energy	25–100	60–130	50–170	>850 MeV/a.m.u.
$\frac{\text{Ca}}{\text{Mn+Fe+Ni}}$	0.67 ± 0.32	0.28 ± 0.14	0.50 ± 0.30	0.12 ± 0.01
$\frac{\text{Ti}}{\text{Mn+Fe+Ni}}$	0.29 ± 0.19	0.09 ± 0.06	0.56 ± 0.35	0.06 ± 0.01
$\frac{\text{Cr}}{\text{Mn+Fe+Ni}}$	0.55 ± 0.27	0.28 ± 0.14	0.60 ± 0.35	0.05 ± 0.008
$\frac{\text{Ca+Ti+Cr}}{\text{Mn+Fe+Ni}}$	1.51 ± 0.50	0.65 ± 0.24	1.63 ± 0.75	0.23 ± 0.02

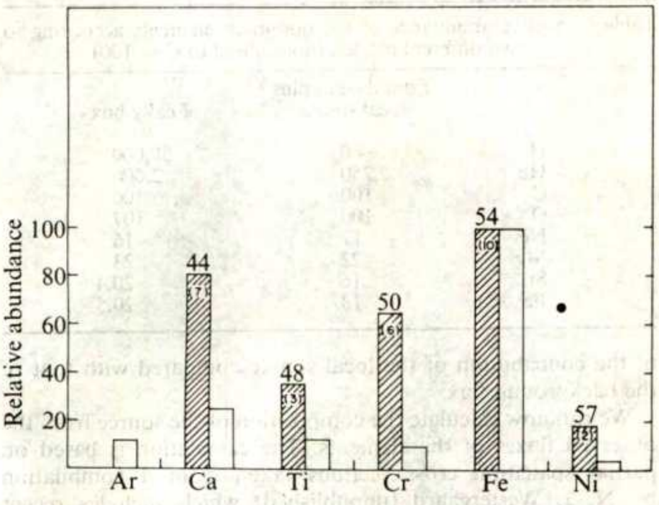
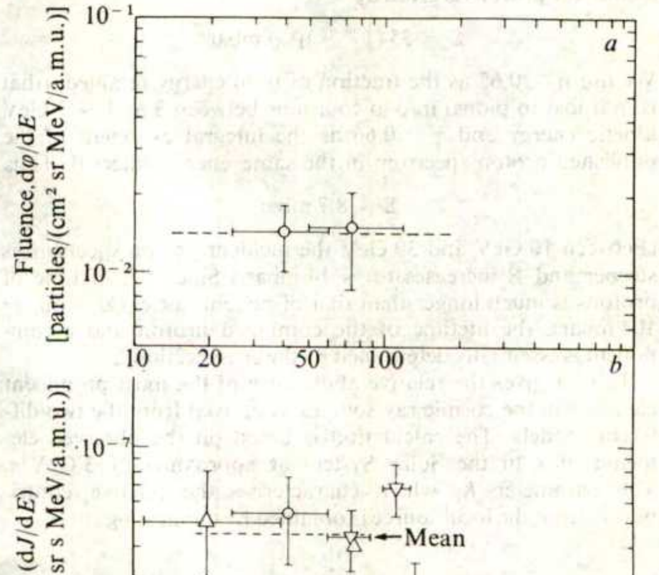


Fig. 2 Relative abundances of Ca–Ni nuclei observed at low energies in the Skylab detector compared with those of high energy cosmic rays. Note the large abundance of Ca, Ti and Cr at low energies. The average energy (in MeV/a.m.u.) and the number of events (in parentheses) are indicated. Shaded columns are our results. Open columns are galactic measurements of Webber *et al.* (> 850 MeV/a.m.u.).

factor for the average interplanetary flux. This is shown by the dotted line in Fig. 3*a*. If we calculate the effective exposure factor for the Skylab detector for the interplanetary flux using the vertical cutoff rigidities derived from the internal geomagnetic field of the Earth[1,8], we obtain the value of $\leqslant 1\%$. The effects of currents in the magnetosphere and the magnetospheric tail were not, however, considered in these calculations. The inclusion of these effects causes a significant lowering in the cutoff rigidity values at locations for which internal field cutoff rigidities are $\leqslant 0.5$ GV, as in the high latitude (±50°) passes of Skylab[8]. Observed fluences shown in Fig. 3*a* can therefore be understood in terms of interplanetary cosmic-ray flux.

Fig. 3 *a*, Differential fluences of Fe-group (Cr–Ni) nuclei measured in the Skylab detector. The estimated fluence at Skylab orbit, based on the average interplanetary flux of these nuclei and an exposure factor of 5% is shown by the dotted line in (*a*). *b*, The observed fluxes of these nuclei in interplanetary space, ▽ represents results of O'Sullivan, *et al.*[2]; ◆ Fleischer, *et al.*[3]; △ Chan, *et al.*[6]; ○ Campero, *et al.*[7].



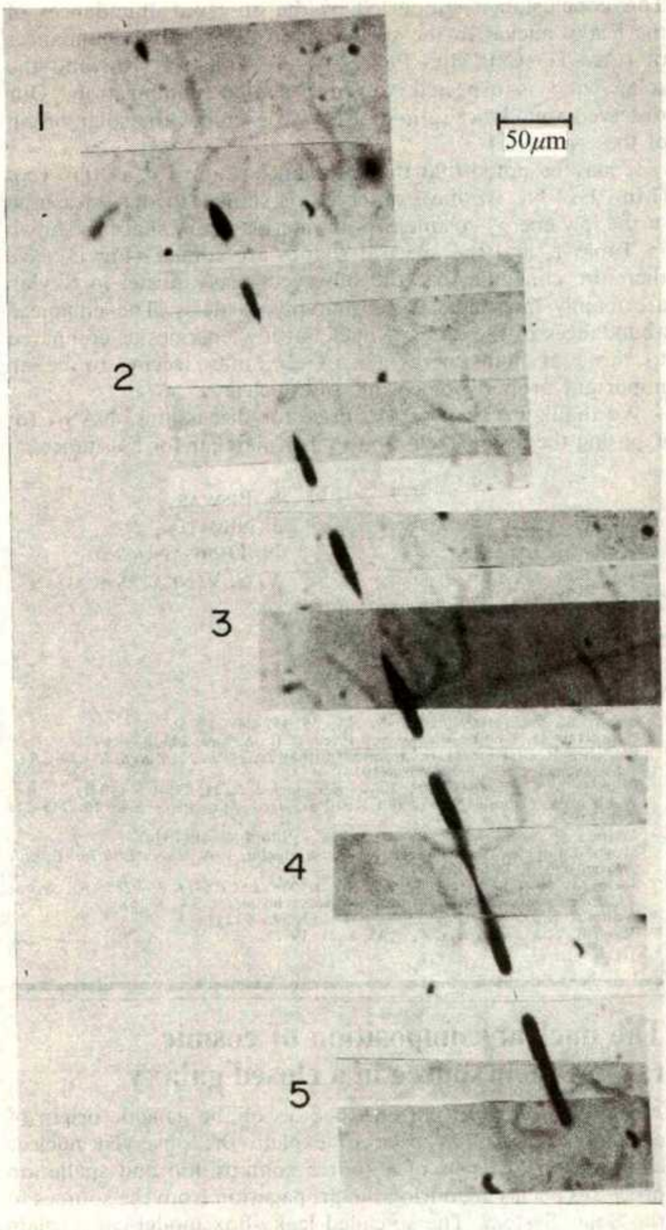


Fig. 4 Mosaic of the photomicrographs of etched tracks produced by an Fe nucleus of energy ~ 60 MeV/a.m.u. in its passage through the top six sheets of lexan detector exposed in Skylab at 435 km altitude for 73 d. The tracks are made visible by etching the sheets after irradiating them with an intense beam of ultraviolet light. Each sheet shows two tracks etched from opposite sides. Note the cones are very small in the top sheet (1). The cones in the topmost sheet (0) are still smaller and not shown in the photograph. The etch cones progressively increase in length as the particle slows down. In sheet (4) the two cones from either side merge together forming a joint double cone. The particle has come to rest in sheet (5). In the *L–R* diagram (Fig. 1) this particle will have as many as six data points making it possible to determine the charge accurately.

Low energy heavy nuclei in the inner radiation belt may originate from solar wind ions of energy ~ 1 KeV/a.m.u. accelerated to a few MeV/a.m.u. in the magnetosphere by the breakdown of the third adiabatic invariant[9]. In this model, the flux of heavy nuclei above ~ 10 MeV/a.m.u. is expected to fall off rapidly. The observed spectrum of Fe-group nuclei in Skylab is almost flat in the energy interval of 25–100 MeV/a.m.u. (Fig. 3*a*). It is therefore unlikely that the trapped inner belt particles contribute significantly to the observed flux.

This conclusion is supported by the observed abundances of the heavy nuclei. In the solar corona the relative abundances of (Ca+Ti+Cr)/(Mn+Fe+Ni) is ~ 0.08 (ref. 10), and the solar wind is expected to have similar composition. Our observed abundance ratio of 1.5±0.5 excludes the solar origin of these particles.

It may be noted that the abundance ratio of (Ca+Ti+Cr)/(Mn+Fe+Ni) we have observed, is similar to that measured in the low energy cosmic rays in interplanetary space as shown in Table 1, and the spectral shapes are similar (Fig. 3). We therefore conclude that the observed heavy nuclei in Skylab are mainly interplanetary cosmic-ray particles. The enhanced abundances of these heavy nuclei at low energies as compared to those at high energy (> 1 GeV/a.m.u.) seems to be an important property of cosmic ray nuclei.

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The nuclear composition of cosmic rays at their source in a closed galaxy

A MAIN objective of current theories of the galactic origin of cosmic radiation has been to explain the observed nuclear composition in terms of a source composition and spallation processes occurring during the propagation from the sources to the Solar System. The so-called leaky-box model can explain the observed composition only if the cosmic rays are assumed to escape from the galaxy after having traversed a few g cm^{-2} of interstellar gas, but has trouble explaining the flux of primaries of large airshowers. These very energetic nuclei are then assumed to be of extragalactic origin, as our own Galaxy cannot contain them long enough to build up the flux to the observed value and to assure even a moderate degree of isotropy.

We construct here an alternative model to explain the observed nuclear composition, which starts with the assumption that all cosmic ray particles are of galactic origin and, once created, remain confined to the galaxy by a suitable large scale magnetic field, even at the highest energies. This implies that the particles are confined also at lower energies, so that all complex nuclei are destroyed by spallation. The particles end up as protons and finally lose their energy due to pion production, a process which requires the traversal of more than 100 g cm^{-2} of interstellar material. We next require that there is one (or more) local sources in the solar neighbourhood (within a distance comparable to the thickness of the galactic disk), contributing nuclei which on their passage to the solar system have traversed only small amounts of matter, which in a first approximation may be neglected. The assumption of a closeby source was first introduced by Lingenfelter[1].

We will further assume that the local source has the same composition as the average of the distant sources. Thus the model contains only one free parameter—the relative strength of the contribution of the local source compared with that of the background flux.

Table 1 Source abundance of the dominant elements according to two different models (normalised to $C = 100$)

	Equilibrium plus local source	Leaky box
H	~0	50,000
He	2,750	2,600
C	100	100
O	100	107
Ne	17	16
Mg	22	23
Si	16	20.4
Fe	13	20.5

We can now calculate the composition of the source from the observed fluxes of the elements. The calculation is based on partial spallation cross sections taken from a compilation by N. J. Westergaard (unpublished) which includes recent results obtained at the Bevalac accelerator

$$S_n = \frac{\sigma_n F_n - \sum_{j>n} \omega_{nj}(F_j - KS_j)}{1 + \sigma_n K} \tag{1}$$

where S_n is the relative source strength for the acceleration of the species n, F_n the observed flux, σ_n the total destruction cross section, ω_{nj} the partial production cross section of species n from species j. K is a parameter describing the overall strength of the local source.

This formula has to be modified for $n \leq 4$, since α-particles as well as protons (or neutrons which decay into protons) occur both as residue in the complete destruction of a complex nucleus and as evaporation particles accompanying its partial destruction.

The corresponding (secondary) flux of nucleons in the combined hydrogen plus helium component from these two contributions is given by

$$\frac{1}{\Sigma}\sum_{i>4}(F_i - KS_i)\left[A_i\sigma_i - \sum_{n>4}^{i-1} A_n\omega_{ni}\right] \tag{2}$$

where A_n is the mass of species n and the attenuation cross section for protons is given by [2]

$$\Sigma = 35\,(1 - \langle\eta^\gamma\rangle)\ \text{mbar}$$

We use $\eta = 0.65$ as the fraction of total energy retained, (that is, not lost to pions) in p–p collisions between 3 and ~ 7 GeV kinetic energy and $\gamma = 0.66$ as the integral exponent of the published proton spectrum in the same energy interval. Thus

$$\Sigma \sim 8.7\ \text{mbar}$$

(Between 10 GeV and 30 GeV the incident proton spectrum is steeper and Σ increases to ~ 14 mbar.) Since the lifetime of protons is much longer than that of helium nuclei ($\Sigma \ll \sigma_{He} = 104$ mbar), the lifetime of the combined proton and α-component is essentially determined by the cross section Σ.

Table 1 gives the relative abundance of the most prominent elements in the cosmic ray sources as derived from the two different models. The calculation is based on the observed elemental flux in the Solar System at approximately 3 GeV/n. The parameter K, which characterises the relative contribution from the local source is obtained by minimising

$$\sum_{m=6}^{11} S_m$$

that is, the amount of Li, Be, and B in the source. This leads to $K = 0.02$ mbar^{-1}. The first column represents the calculated source composition as derived from the present model. The second column represents the composition as derived by

Shapiro *et al.*[3]. Their calculation is based on the leaky-box model in which the cosmic rays are allowed to escape from the galactic disk after having traversed a few g cm^{-2} of interstellar gas. The path length distribution is assumed to be a truncated exponential. The 'equilibrium plus local source' model differs from the leaky-box model mainly in that protons are not emitted by the cosmic ray sources, but are disintegration products of the heavier nuclei.

The local source accounts for a fraction f of the observed flux, given by

$$f = \frac{K\sigma_n}{1+K\sigma_n};$$

it differs for each primordial element:

$$f = \left\{\begin{array}{l} 93.4\% \text{ for Fe} \\ 88.6\% \text{ for Mg} \\ 85.3\% \text{ for O} \\ 67.5\% \text{ for He} \\ 0\% \text{ for H} \end{array}\right.$$

This means that it is responsible for about 15% of the incident nucleons of a given velocity around 3 GeV/n. Thus, the present model predicts some anisotropy for the heavy nuclei, but probably not enough to disturb significantly the overall isotropy of cosmic radiation in the Solar System.

The 'equilibrium plus local source' model can account for the observed fluxes of all those elements which are assumed to be present in the source as well as for the observed flux of H, Li and Be. It predicts somewhat too little B and elements in the sub-iron group, that is with atomic numbers between 19 and 25.

A first step in refining the model is to assume that the particles from the local source have traversed on the average not zero but a small amount of matter before reaching the Solar System. This introduces a second free parameter into the model. The importance of the local contribution suggests that this matter is located in the source itself, or in its neighbourhood, and that, therefore, the path lengths are faily narrowly distributed around a mean value x which probably decreases with increasing particle rigidity. The correct ratio between the observed flux of iron and elements of the sub-iron group at 3 GeV/n requires $x \sim 1$–2 g cm^{-2}, and the expected decrease of x with increasing rigidity would account for the observed increase of this ratio with energy.

The discrepancy between the predicted and observed flux of Boron suggests that particles from the local source have perhaps undergone some processing by spallation reactions, which favour the removal of a single nucleon from a heavy nucleus, such as a strong irradiation by γ rays, or possibly some interactions in the source region during the early stage of the acceleration process.

The observed energy dependence of the ratio between light nuclei (Li, Be, B) and medium nuclei (C, N, O) seems easily reconcilable with the model, if one assumes that the spectrum of the local source is slightly flatter than that of the average of the distant sources. An important prediction, if one accept this explanation, is that the proton spectrum should be steep and follow that of light nuclei, rather than C, N, and O, because the protons are not primordial but secondary.

General arguments make it seem likely that the model can account for the observed isotopic abundances of H, He, and Li; and that ^{10}Be should be practically absent at low energies, in agreement with data recently obtained by the Chicago group and others. In contrast, some shorter-lived radioactive isotopes could be present if they come from the local source. The observed positron flux might be reconciled with the model using acceptable values for solar modulation parameters and for the gas and field density of the galactic confinement volume, which necessarily will be much larger than the volume of the galactic disk. The expected γ-ray flux is not changed, since it depends on the flux of nucleons in the galaxy, not on their lifetime.

Predictions, based on this model at energies below the range considered here, will have to take into account the large difference in energy loss by ionisation of the old and the new particles in the observed flux.

The main advantage of the model seems to us to be that it makes a galactic origin of cosmic rays possible at all energies, and thus abolishes the need for the extragalactic cosmic ray component which was supposed to dominate in the very high energy region. It gives a reasonable account of the curious ratio of hydrogen to complex nuclei in the cosmic radiation which differs from the ratio in interstellar matter and also from the ratio in ordinary stars and on the surface and in the interior of supernovae. Also the total amount of energy necessary to maintain the cosmic ray intensity in the galaxy is appreciably reduced, since it will no longer be necessary to replenish the entire flux every ~ 3 Myr.

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Mantle-plume model for the origin of Archaean greenstone belts based on trace element distributions

TRACE element studies of Archaean greenstone volcanics indicate the existence of two major types of tholeiite[1–3]: depleted Archaean tholeiite (DAT) showing flat rare earth element (REE) patterns similar to modern rise tholeiites and enriched Archaean tholeiite (EAT) exhibiting slight light REE enrichment and generally greater concentrations of LIL (large-ion lithophile) elements than DAT. Included in the DAT category are komatiitic tholeiites. DAT and associated ultramafic flows and sills seem to make up from 50 to 90% of Archaean greenstone successions. EAT becomes relatively more abundant at stratigraphically higher levels and in the upper parts of minor volcanic cycles within greenstone successions. Although overall EAT is generally less abundant than DAT, it accounts for up to 50% of some greenstone sections[2,4]. DAT seems to be absent in the upper 10–20% of most greenstone successions.

In terms of trace element distributions, andesites in Archaean greenstone belts fall into three categories[1–3]: depleted Archaean andesite (DAA) exhibits flat REE patterns, negative Eu anomalies and low LIL-element content; low-alkali Archaean andesite (LAA) shows minor light REE enrichment and low LIL-element content; and high-alkali Archaean andesite (HAA) shows light REE enrichment and high LIL-element contents. Andesites are minor in most greenstone belts (western Kenya being an exception[4]), becoming relatively abundant only in the upper 30% of greenstone successions. HAA exceeds LAA in relative importance only in the upper 10% of most sections. DAA has so far only been reported from the Abitibi belt in Quebec where it is associated with DAT also exhibiting negative Eu anomalies[1].

Siliceous volcanic rocks (dacite, rhyodacite, rhyolite) in Archaean greenstone belts fall into two groups based on trace element contents: depleted siliceous volcanics (DSV) exhibit heavy REE and Y depletion, whereas undepleted siliceous volcanics (USV) do not. Both groups exhibit en-

Table 1 Summary of trace element models for the origin of Archaean greenstone and granite magmas

Tholeiite	
DAT	25–35% melting of lherzolite or 10–20% melting of plagioclase peridotite
EAT	45–55% melting of eclogite
Andesite	
DAA	10–20% melting of plagioclase peridotite
LAA	20–30% melting of eclogite
HAA	10–20% melting of eclogite
Siliceous rocks	
DSV and tonalite	10–20% melting of eclogite
USV and granodiorite	30–60% melting of siliceous granulite
High-K granite	70–80% fractional crystallisation of granodiorite

From data in refs 1–4, 7 and 8.

richment in transition metals when compared with younger analogues. Siliceous volcanics, in general, make up only a minor amount of the greenstone successions (< 10%) and are often restricted (with the exception of dikes) to the upper 10–20% of the section. If existing analyses are representative, DSV exceeds USV in abundance. Greenstone belts are intruded by a variety of granitic plutons. Although such granitic rocks are not adequately described in most areas, they are reasonably well known in the Barberton region of South Africa[5,6]. In this area, the oldest intrusions are tonalite diapirs followed by voluminous granodiorite batholiths and finally by minor, anorogenic high-K granites. The tonalites have trace element patterns similar to DSV and the granodiorites to USV. The high-K granites are characterised by high LIL-element content and negative Eu anomalies.

Trace element studies have recently proved valuable in testing various mechanisms of partial melting and fractional crystallisation for the origin of the magmas that produced the igneous rocks in greenstone-granite terrains[1,2,4,7,8]. Relying chiefly on those trace elements least susceptible to mobilisation during alteration and low grade metamorphism (namely, Y, Zr, REE, Co, Ni and so on), it is possible to eliminate many models for magma origin and to focus on others. Although fractional crystallisation models are allowed or necessitated in some instances[7,8], crystal–liquid equilibria during partial melting seem to have left the strongest imprint on trace element distributions in greenstone volcanics and associated granitic rocks. The most acceptable trace-element models for each of the volcanic and granitic rock types found in Archaean greenstone belts are summarised in Table 1.

Any model for the origin and development of Archaean greenstone belts and associated granitic terrains must account for the following features: (1) the dominance of an ultramafic magma source area during the early and intermediate stages of greenstone belt evolution; (2) the increasing importance of an eclogite source area during the later evolutionary stages; (3) partial melting of siliceous granulite during the late stages to produce granodiorite and high-K granite magmas; (4) an overall decreasing degree of partial melting in the source regions with time, and (5) the origin of minor volcanic cycles which seem to necessitate replenishment of both ultramafic and eclogite sources.

One model that seems capable of explaining these features is a mantle-plume model[8], the overall features of which are described here. A mantle plume with a region of extensive melting at its top ascends, penetrates the lithosphere, and spreads laterally producing an overlying rift system which is partially filled with DAT and ultramafic magmas derived from the plume. It is not critical to the model whether earlier sialic crust existed or not. A large volume of these magmas are intruded into the lithosphere above and adjacent to the plume and, because of the steep geothermal gradient, the mafic magmas crystallise into garnet granulite mineral assemblages. As the plume begins to cool and subside, the overlying lithosphere begins to sink. A decreasing geothermal gradient results in the inversion of garnet granulite above the plume to eclogite. Continued sinking of the eclogite results in its partial melting giving rise to EAT magmas and, with a further decrease in temperature, to minor volumes of andesite and more siliceous magmas. Some of the latter rise as tonalite diapirs. Further sinking in response to plume subsidence leads to partial melting of siliceous granulites in the upper part of the lithosphere (lower crust) thus producing granodiorite magmas and, through fractional crystallisation, high-K granite magmas. The siliceous granulites may represent the lower portion of an older sialic crust upon which greenstone belts erupted and/or they may have crystallised from magmas of intermediate composition emplaced at appropriate depths in the lithosphere overlying the plume.

Thus the plume model provides the necessary changing source region composition for magma generation with time as well as a dropping geothermal gradient. Minor volcanic cycles within greenstone belts can be produced by cyclical plume activity, with each cycle decreasing in intensity with time. Renewed plume activity during a cycle replenishes the DAT source (directly from the plume) and, by intrusion and later recrystallisation of DAT magma, the eclogite source. It should be pointed out that the flat REE patterns and negative Eu anomalies in mafic and intermediate volcanics from the Abitibi belt may require a plagioclase peridotite source[1,3]. In any case, eclogite is not an acceptable source rock for any of the volcanics analysed from this belt.

The linear arrays of greenstone belts in some areas (such as the Superior Province) and irregular distribution in others (such as Rhodesia) may have developed in response to plume patterns which in turn reflect differences in Archaean structural inhomogeneities in the upper mantle. Variations in the relative abundances of volcanic rocks in greenstone belts in different geographic locations[3,4] can also be accounted for by a plume model. Large, relatively hot plumes and/or a thin lithosphere would favour production of greenstone successions in which DAT greatly dominates, such as the Barberton and greenstone belts in western Australia. Small, relatively cool plumes and/or thick lithosphere (especially if composed largely of eclogite) on the other hand, would favour the production of relatively greater volumes of EAT, intermediate, and siliceous magmas such as characterise the Midlands belt in Rhodesia and the greenstone belts in western Kenya.

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Phytenic acid in sediments

PHYTANIC acid and several lower isoprenoid acids are found in the marine food chain and in sediments. The mechanism of the degradation of phytol to phytanic acid is still uncertain. At least two pathways have been proposed and shown to occur in some cases[1]. One by way of dihydrophytol, another by phytenic aldehyde and phytenic acid. Phytenic acid was postulated recently[2] as intermediate in the degradation of phytol in sediments and was mentioned among isoprenoid compounds in Mud Lake sediment[3]. It is formed from phytol thermocatalytically in oxic conditions[4].

We present and discuss the occurrence of phytenic acid in a lacustrine detritus layer and in a marine diatomaceous ooze. Since the analytical behaviour of the acid is peculiar, care must be taken that its presence is not overlooked. Therefore chemical and chromatographic data are discussed at length.

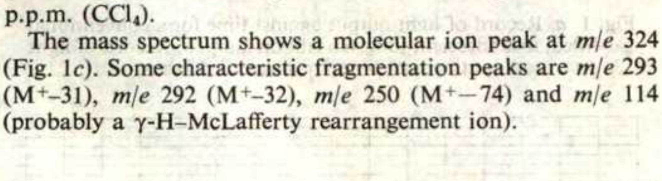
The lacustrine detritus predominantly consists of remains of *Fontinalis antipyretica* in an apparent state of degradation. It was sampled from Lonnekermeer (Twente, Holland)[4] which is a small lake considered to be in a non-polluted state. The wet detritus was extracted with chloroform and the extract subjected to silica-gel column chromatography, using gradient elution with pentane–ethyl acetate. The acid fraction was methylated[5] and the resulting methyl esters were separated into saturated, mono-unsaturated and poly-unsaturated fractions by means of preparative $AgNO_3$-thin layer chromatography (TLC) (10% $AgNO_3$ on silica gel), using hexane–diethyl ether (97.5:2.5 v/v) as an eluent. Methyl phytenate was shown to be present in the saturated methyl ester fraction as indicated by its gas–liquid chromatography (GLC)-retention time and mass spectrum, obtained on a Varian MAT 111 GC/MS instrument using a 30-m capillary column (0.25 mm inner diameter) coated with SP 2250. Helium was used as carrier gas.

The marine sediment is a diatomaceous ooze from the inner shelf of S.W. Africa (Walvis Bay). Four sediment sections were investigated (B41: 3–28 cm; B16: 28–53 cm; B26: 53–78 cm; B1: 78–103 cm) from a core KF6 (23°15′S and 14°05.5′E). The sediment was handled as described earlier[6]. Freeze-dried sediment was extracted with 0.1 N KOH in 95% methanol–benzene (4:1 v/v)[7]. After acidification a total lipid extract was obtained. After selective extraction of free fatty acids from the extract[8], the neutral fraction was separated by preparative thick layer chromatography on silica gel[9]. Methyl phytenate was present in the TLC-cut containing the aromatic hydrocarbon fraction (see Table 1).

Phytenic acid was found to be present in the four sediment sections of core KF6. No phytenic acid was produced from phytol during the experiment, as was shown by applying the complete analysis to a sample of pure phytol.

Phytenic acid was synthesised from purified natural phytol (Merck), by subsequent oxidation with MnO_2 and Ag_2O[10]. The reaction mixture was methylated[5] and methyl phytenate was isolated by preparative thick layer chromatography on silica gel[9]. GLC analysis indicated the presence of one methyl ester (ECL value 18.45). Infrared measurement shows a carbonyl absorption at 1,720 cm^{-1} (CCl_4) characteristic of an α,β-unsaturated ester, and an absorption of 1,650 cm^{-1} which points to a substituted double bond conjugated with a carbonyl function. Nuclear magnetic resonance (NMR) measurement (Varian H60) reveals a signal for the olefinic proton at δ 5.6 p.p.m. (CCl_4).

The mass spectrum shows a molecular ion peak at *m/e* 324 (Fig. 1*c*). Some characteristic fragmentation peaks are *m/e* 293 (M^{+}−31), *m/e* 292 (M^{+}−32), *m/e* 250 (M^{+}−74) and *m/e* 114 (probably a γ-H–McLafferty rearrangement ion).



These data agree well with the structure of (E)-3,7,11,15-tetramethyl hexadec-2-enoic acid methyl ester. The chromatographic data of this compound presented in Table 1, were obtained by silica-gel TLC[9] and by $AgNO_3$-TLC on silica gel using hexane–diethyl ether (97.5:2.5 v/v).

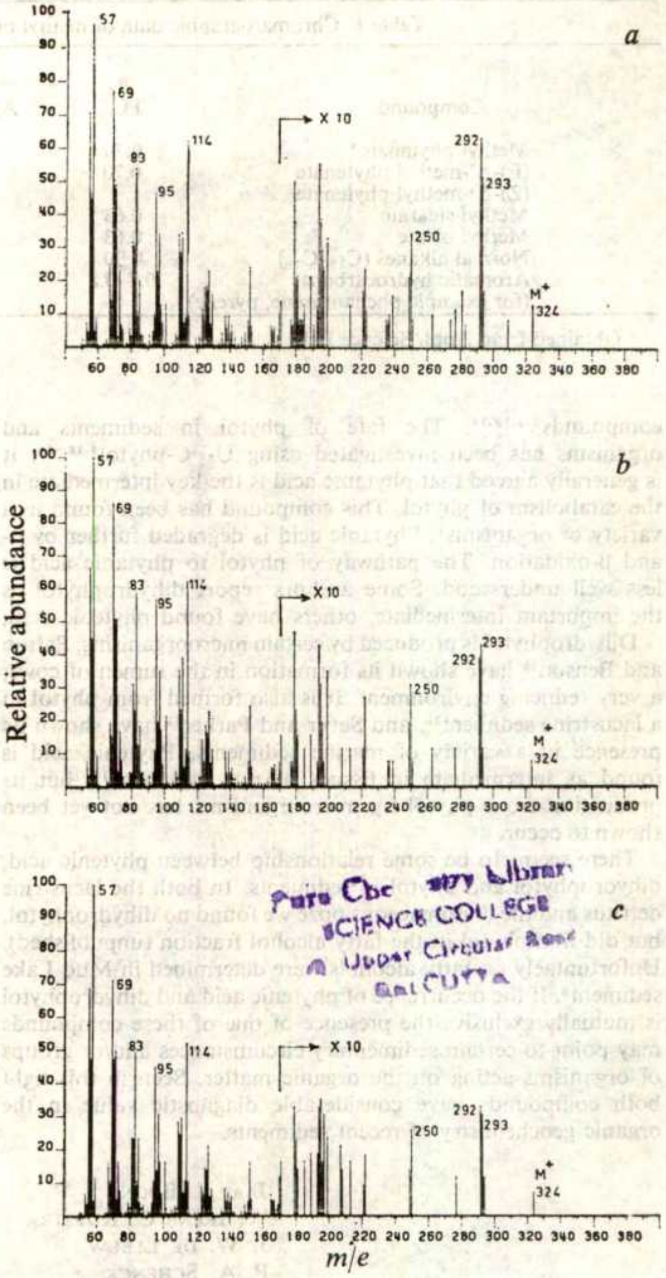


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Fig. 1 Mass spectra of phytenic acid methyl ester. *a*, From lacustrine detritus; *b*, from marine diatomaceous ooze; *c*, synthetic standard compound

Figure 1 presents the mass spectrum of the methyl ester from both sediments tentatively identified as methyl phytenate, and the mass spectrum of the standard, which prove to be almost identical. Because of the peculiar chromatographic behaviour (see Table 1), it becomes clear that definite identification can only be based on the mass spectral data obtained by GC/MS.

Use of $AgNO_3$-TLC and GLC methods only, can easily lead to a misinterpretation of the methyl phytenate peak which is incorrectly considered a saturated ester. Another hazard is the apparent ease of esterification of phytenic acid. If alkaline alcoholysis is used to obtain the fatty acids from sedimentary, plant or animal material, the phytenic acid shows up in the neutral fraction as the ester (see Table 1).

It is known that esters can easily be formed when sediments are extracted with methanol containing solvents in the presence of clay surfaces[12]. In our case the TLC and $AgNO_3$-TLC data, the equivalent chain length (see Baxter[11]) and the mass spectrum both indicate that for both sediments we have (E)-3,7,11,15-tetramethyl hexadec-2-enoic acid. This compound can be seen as an intermediate in the conversion of phytol to other isoprenoid

Table 1 Chromatographic data of methyl phytenate as compared with standard compounds

Compound	R_f TLC	R_f Ag^+–TLC	R_f Ag^+–TLC[11]	ECL value SP 2250	Relative retention time SE 30[11]
Methyl phytanate*	0.70	0.29	0.53	1,722	1.00
(E)-Δ^2-methyl phytenate	0.70	0.27	0.53	1,845	1.56
(Z)-Δ^2-methyl phytenate			0.62		1.07
Methyl stearate	0.63	0.22	0.47	1,800	1.12
Methyl oleate	0.63	0.09	0.26		
Normal alkanes (C_{10}–C_{36})	0.90				
Aromatic hydrocarbons (for example phenanthrene, pyrene)	0.7–0.8				

* Obtained from Appl. Science Lab.

compounds[2,4,13,14]. The fate of phytol in sediments and organisms has been investigated using U-^{14}C-phytol[4,14–16]. It is generally agreed that phytanic acid is the key intermediate in the catabolism of phytol. This compound has been found in a variety of organisms[1]. Phytanic acid is degraded further by α- and β-oxidation. The pathway of phytol to phytanic acid is less well understood. Some authors report dihydrophytol as the important intermediate, others have found phytenic acid.

Dihydrophytol is produced by certain microorganisms. Patton and Benson[15] have shown its formation in the rumen of cows, a very reducing environment. It is also formed from phytol in a lacustrine sediment[16], and Sever and Parker[17] have shown its presence in a variety of marine sediments. Phytenic acid is found as intermediate in tissues of rats and mice[14], but its production from phytol by microorganisms has not yet been shown to occur.

There seems to be some relationship between phytenic acid, dihydrophytol and phytol in sediments. In both the lacustrine detritus and the diatomaceous ooze we found no dihydrophytol, but did find phytol in the fatty alcohol fraction (unpublished). Unfortuntaely no fatty alcohols were determined in Mud Lake sediment[3]. If the occurrence of phytenic acid and dihydrophytol is mutually exclusive the presence of one of these compounds may point to certain sedimentary circumstances and/or groups of organisms acting on the organic matter. Seen in this light both compounds have considerable diagnostic value in the organic geochemistry of recent sediments.

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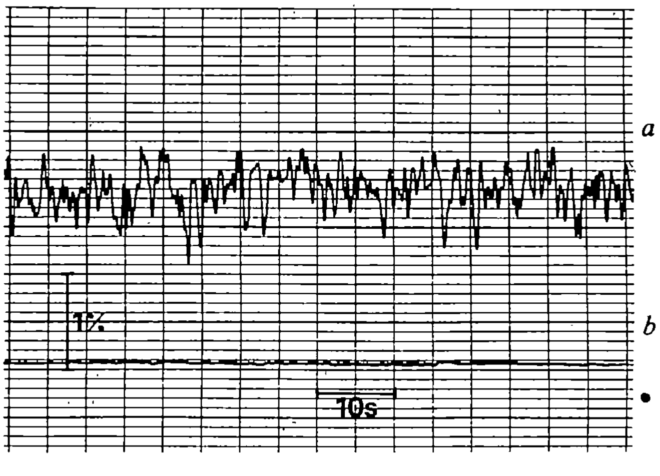
Fluctuations in the light output from gas-filled incandescent lamps

THE gas-filled, incandescent tungsten lamps used as photometric and radiometric standards have good long term stability, but their performance is frequently marred by fluctuations in light output resulting from changes in the convective cooling of the filament. Typical fluctuations are shown in Fig. 1*a*, which is a record of the output from a ribbon filament lamp of the type used at the National Physical Laboratory (NPL) as a standard of spectral radiance (Fig. 2*a*). In this situation, an accuracy of 0.1% (a common requirement in standards work) can only be obtained by averaging for several seconds, a tedious restriction where a large number of measurements is required.

It is not possible to dispense with the gas filling without incurring excessive evaporation of tungsten from the filament, but the fluctuations might be reduced if the convective gas flow within the lamp could be stabilised aerodynamically. We therefore studied the pattern of the gas flow by the use of a Schlieren system in conjunction with a number of special lamp envelopes having optically flat windows. To facilitate quick and easy access for modification inside the lamp, the envelopes were left unsealed and made demountable, the experiments being carried out in air at atmospheric pressure. As tungsten would have oxidised in these conditions, filament models were made from platinum or palladium and operated at relatively low temperatures, below their melting point. A number of different structures were examined and it was found that the fluctuations were effectively suppressed by the provision of a cylindrical chimney some 25 mm in diameter and 60 mm long mounted

Fig. 1 *a*, Record of light output against time for a conventional gas-filled, incandescent, tungsten ribbon lamp. *b*, Record of light output against time for a modified lamp.



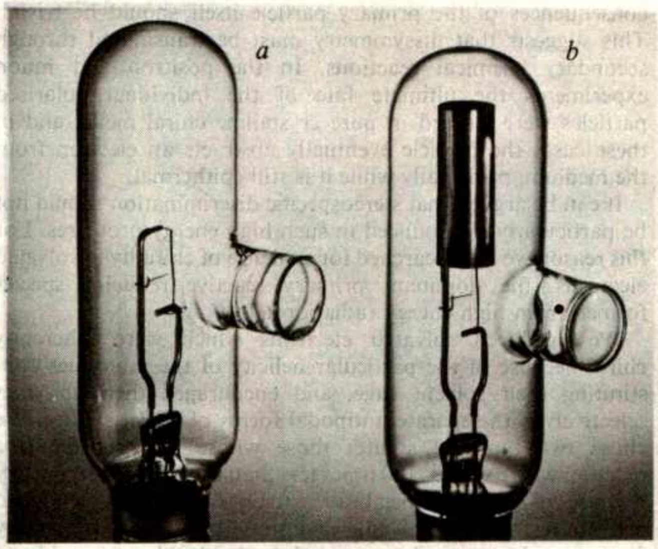


Fig. 2 *a*, Gas-filled, incandescent, tungsten ribbon filament lamp used at the NPL as a standard of spectral radiance. *b*, The lamp modified by the addition of a cylindrical chimney above the filament.

vertically just above the top of the incandescent filament. It was, of course, necessary to confirm these findings in actual lamps, and a number of argon-filled, tungsten ribbon lamps of the conventional NPL pattern, but incorporating the chimney, were made to our specification by the Hirst Research Laboratory of the General Electric Company (Fig. 2*b*). The effect of the modification on the light output is shown in Fig. 1*b*.

Our Schlieren observations showed that in the unmodified lamp, the plume of hot gas which rises from the base of the filament tends to wander across the filament in a manner reminiscent of a guttering candle flame. The chimney seems to separate the central rising gas column from the cooler gases flowing back down the outer parts of the lamp envelope, giving smooth and stable gas flow in the region of the filament. The dimensions of the cylinder did not seem to be critical for the lamp investigated and it seems likely that the fluctuations in other types of gas-filled incandescent lamps could be suppressed in a similar way.

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Preliminary palaeoenvironmental implications of pollen analysis of Middle Breccia from Sterkfontein

SUCCESSIVE excavations at the Sterkfontein site, about 50 km west of Johannesburg, have yielded numerous vertebrate remains, including those of *Australopithecus* and possibly *Homo*, together with some artefacts. The geology and stratigraphy of Sterkfontein have been described by Butzer[1] and by Partridge[2], and Vrba[3,4] has discussed the chronological and palaeoecological implications of the fossil mammals found there.

Robinson[5] divided the sequence into a Lower Breccia, later referred to as the STS unit, containing vertebrates and *Australopithecus*, a Middle Breccia containing possible *Homo* and numerous artefacts, and an Upper Breccia. T. C. Partridge (personal communication) suggested recently that the entire sequence should be included under the term "Sterkfontein Formation", divided into a number of members and beds. The Sterkfontein Formation sediments comprise red breccias and siltstones, held together by travertine which is most probably a secondary cement.

The relative scarcity of pollen grains in these sediments made earlier attempts at extracting them difficult, whereas the high carbonate content and the red coloration apparently prevented any later attempts.

The pollen extraction described here was based on the idea that the sediments had been washed into a previously existing cave after having been oxidised outside the cave, and that the travertine represents a later stage of carbonate precipitation that could not interfere much with the pollen preservation. This was also encouraged by positive results obtained while analysing cave deposits from Israel.

A sample of ~ 2.5 kg of sediment was collected from the Middle Breccia (unit SE, or member 5 of the Sterkfontein Formation), and underwent a long process of preparation. Basically this process involved total dissolution of the carbonates with 10% HCl, partial dissolution of the silicates and clays with a 40% HF, and subsequent mineral gravitational separation using $ZnCl_2$ solution in 10% HCl, with a specific gravity of 1.9. The residue was washed with 10% KOH and water and mounted in glycerine jelly. The results were surprising, and thousands of pollen grains were recorded in the slides. For comparison, a sample of recent soil from the site was also prepared.

About 400 pollen grains have been counted in the slides prepared from the Middle Breccia sample, and were divided into two groups on the basis of their state of preservation (Fig. 1): the first assemblage, which yielded 138 grains, comprised heavily corroded and carbonised fossils. The second assemblage, which yielded 262 grains, comprised only slightly corroded and

Fig. 1 Comparison between badly and better preserved pollen from the Middle Breccia, Sterkfontein (×930). *a*, *b*, Gramineae. *c*, *d*, *Podocarpus* spp. *e*, *f*, Compositae.

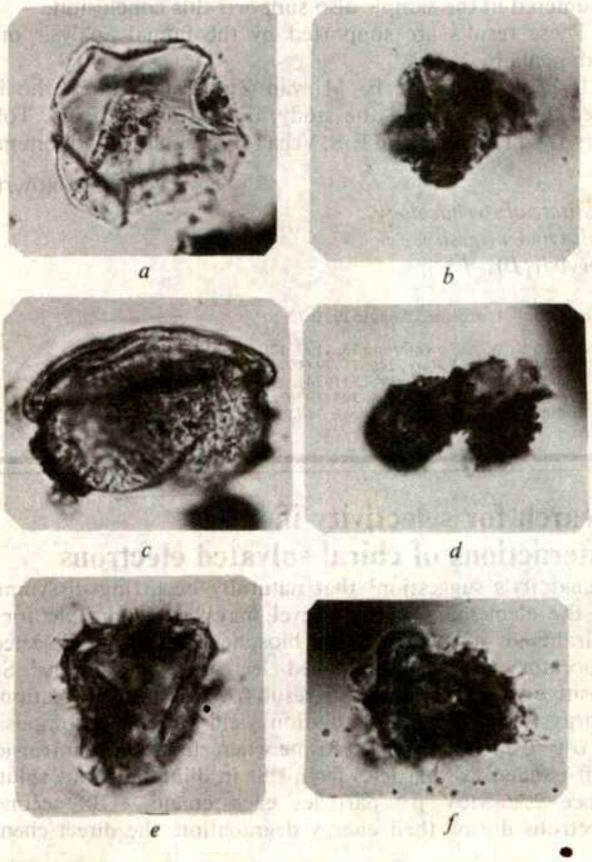

Table 1 Pollen spectra of samples from Sterkfontein (percentage of the total for each group)

	Middle Breccia		Recent
	Badly preserved	Better preserved	
Gramineae	84	48	37
Compositae	11	5	2
Fern spores	3	9	1
Podocarpus	1	10	1 (other than fossils)
Euphorbia		16	
Cyperaceae		3	12
Rhus		4	33
Pinus			2
Olea			5
Chenopodiaceae		1	5
Unidentified	2	3	1
Others		3	1

carbonised material. The Recent material was much better preserved. Table 1 summarises the percentages of the first and second groups, in comparison with the Recent spectrum.

A preliminary suggestion is hereby proposed as to the palaeoenvironmental implications of the two pollen spectra from the Middle Breccia: the badly preserved, apparently older material, represents the environment at the time of accumulation of the cave sediments, including the bones and artefacts, whereas the second, better preserved material was brought into the sediments by the percolating water during a later stage, a process that has been responsible for the travertine deposition and part of the secondary cementation.

Each of the two spectra is totally different from the Recent one. It is suggested, based on the predominance of Gramineae pollen and the scarcity of other species, that the Middle Breccia was formed when the surrounding area was a vast, open grassland, and probably when the climate was drier than at present. The travertine formation took place when the climate was much more humid, and probably also warmer, than at present. This is indicated by the *Podocarpus* and *Euphorbia* assemblage that appears today towards the lower, warmer and more humid areas of the Transvaal. The single grain of *Rauvolfia* that was encountered in the sample also supports this conclusion.

These results are supported by the faunal analysis of the sediments by Vrba[3,4].

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Search for selectivity in interactions of chiral solvated electrons

ULBRICHT's suggestion[1] that naturally occurring dissymmetry at the elementary particle level may be responsible for the chirality of molecules in the biosphere has been subjected to laboratory tests using polarised electrons[2,3] and muons[4]. Some positive results are claimed to result from β^- and β^+ radiations[2,3]. Garay found a higher radiation yield for the decomposition of D-tyrosine than for L-tyrosine when subjected to intrinsically 'left-handed' β^- particles from ^{90}Sr in dilute aqueous solution. Since 2.26-MeV β^- particles each create $\sim 10^5$ secondary electrons during their energy degradation, the direct chemical consequences of the primary particle itself should be trivial. This suggests that dissymmetry must be transmitted through secondary chemical reactions. In the positron and muon experiments the ultimate fate of the individual polarised particles were studied in pure crystalline chiral media and in these cases the particle eventually abstracts an electron from the medium, principally while it is still epithermal.

It can be argued that stereospecific discrimination should not be particularly pronounced in such high energy processes. For this reason we have searched for evidence of chirality in solvated electrons—the dominant primary reactive reducing species formed from high energy radiations.

We produced solvated electrons which were inherently chiral because of the particular helicity of the molecules constituting their solvent cage, and encouraged them to react selectively with separate antipodal forms of chiral solutes. We chose two types of solute: those whose rate constant for reaction with e was $\sim$ 100 times less than the collision frequency because of stereochemical, or entropy, factors (that is, no activation energy involved); and those with high asymmetry factors, such as camphor. L$^-$ and DL-3-chlorobutyric acid was used as the solute of the former kind since it had $k \sim 10^8$, and the ractive site—the Cl atom—was at the asymmetric centre. We also looked for optical activity in these systems.

Just as the interaction of right-circularly polarised light is different for the D and L enantiomers of a chiral molecule, so it is supposed here that an electron confined to a right-handed helical solvent may show a different probability of reaction with D and L enantiomers: that is, it is supposed that, of the four reaction rate constants, $k_1 \neq k_2$ and $k_3 \neq k_4$, whereas $k_1 = k_3$ and $k_2 = k_4$.

$$e_L + s_L \xrightarrow{k_1} \qquad (1)$$

$$e_D + s_L \xrightarrow{k_2} \qquad (2)$$

$$e_D + s_D \xrightarrow{k_3} \qquad (3)$$

$$e_D + s_L \xrightarrow{k_4} \qquad (4)$$

where e_D and e_L are electrons solvated in right- and left-handed chiral molecules and s_D and s_L are D and L solutes.

The search for a difference in reaction rates between alike (reactions (1) and (3)) and unlike (reactions (2) and (4)) has been pursued by comparing the absolute rate constants obtained in separate experiments for the reaction of the same chiral solute in the D and L (or DL) solvent, and by scrutinising the decay curve of racemic solvated electrons when they react with a chiral solute to see if there is evidence for more than one reaction rate. (Racemic mixtures of solvated electrons would be present in a DL solvent—some environments being intrinsically symmetric, the rest being 50 : 50 mixtures of dissymmetric forms—or in a pure chiral solvent where the $+\frac{1}{2}$ and $-\frac{1}{2}$ spin states would be enantiomers.)

The experiments were carried out by pulse radiolysis using the 3-ns pulse of 600-kV electrons of a Febetron 706 accelerator. Concentrations of solvated electrons were monitored by kinetic laser photometry at 633 nm (refs 5 and 6). As chiral media we used L-2-methyl-1-butanol, D-(and DL)-2-butanol and (β) 5 M sucrose in water—for which induced chirality may be anticipated[7]. Solvated electrons in these solvents had broad structureless bands centred at $\sim$ 700 nm. High solute concentractions ($> 10^{-2}$ M) were always used to ensure pseudo-first order decays in (e) and to limit the electron lifetime to $< 10^{-7}$ s to minimise spin flipping.

The sum $(e_L) + (e_D)$ was determined by the measured absorbance at time t (A_t) in the kinetic studies on racemic solvents, such that

$$A_t = A_0\{\exp(-k_a t)+\exp(-k_b t)\}/2 \qquad (5)$$

where A_0 is the absorbance at the end of the pulse ($t = 0$) and k_a and k_b are the pseudo-first order rate constants for e_L and e_D with s_L. Whenever there is non-preferential reactivity (a negative result) equation (5) reduces to a simple exponential. All the data were analysed on the basis of equation (5) through a computer fit of decay curves to give the best values of k_a and k_b.

No significant difference between k_a and k_b was found when the experiments were repeated many times using L-3-chlorobutyric acid in 2-methyl-1-butanol or L-camphor in 2-butanol. In 70% of the experimental runs involving the racemic (and achiral) solvents the analysis gave $k_a = k_b$, and the difference $|k_a - k_b|$ was not more than 10% in a significant number of cases. (In fact the decay rate during the first half life was not consistently greater than that during the second half life.) Similar results were obtained for the pure chiral solvents; k_a and k_b were found to be equivalent for at least 75% of the time.

In the search for transient optical activity we used a plane-polarised analysing light beam coupled with a crossed linear analyser before the detector. In these conditions no light reached the detector—unless the plane of polarisation was rotated on traversing the cell because of the production of unequal concentrations of e_L and e_D with dissimilar refractive indices for left- and right-circularly polarised light. No such optical rotation was found in any of these systems, even when the sensitivity of the detector was considerably increased.

In summary, we found no evidence for preferred reaction (differences in rate constant < 10%) and were also unable to detect optical activity of solvated electrons in a pure chiral solvent (or in an achiral solvent containing large quantities of an unreactive chiral solute, 5 M sucrose in water) or at any stage during the decay of a racemic mixture of electrons which react with chiral solutes. Two closely related studies should be mentioned. First, tests for circular dichroic spectra of stable trapped electrons in low temperature glasses have established an upper limit of 3×10^{-3} for the asymmetry factor (g) for these solvated electron antipodes (R. May, L. D. Hayward and D.C.W., unpublished data). Second, when DL-camphor was extensively γ irradiated (using ^{60}Co γ rays, which are circularly polarised) in a chiral solvent (D-2-octanol) no preferred destruction of one enantiomer of the camphor could be detected through the development of optical activity[8].

Apparently a rather subtle selectivity must be involved if asymmetry in nature was introduced from dissymmetry at the elementary particle level through the indirect action of the radiation.

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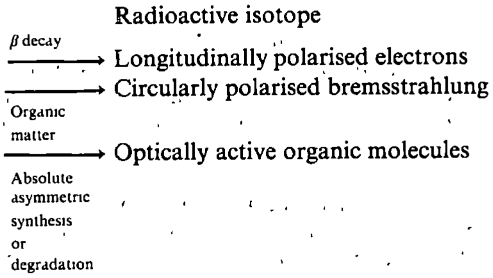
Asymmetric degradation of DL-leucine with longitudinally polarised electrons

FOLLOWING Wu's experimental demonstration[1] of Lee and Yang's predicted violation of the parity principle during β decay[2] and following Goldhaber's demonstration[3] of the circular polarisation of the bremsstrahlung produced by β-decay electrons, Vester and coworkers (ref. 4 and F. Vester, unpublished) proposed the following unique mechanism for the abiotic origin of optically active organic molecules


Radioactive isotope
β decay
Longitudinally polarised electrons
Circularly polarised bremsstrahlung
Organic matter
Optically active organic molecules
Absolute asymmetric synthesis or degradation

Attempts to demonstrate this mechanism experimentally[4,5] were unsuccessful, however, until Garay's report in 1968 that D-tyrosine in dilute aqueous alkali was more decomposed (as evidenced by greater eradication of its ultraviolet absorption bands) than was L-tyrosine after 18 months exposure to 0.36 mCi of ^{90}SrCl$_2$ in solution[6]. One of us has attempted[7] to repeat Garay's experiment using DL-tyrosine in alkaline solution, looking for optical rotation after exposing the sample to a dose of 4.1×10^8 rad of β-ray bremsstrahlung in a 61,7000-Ci ^{90}Sr–^{90}Y source at Oak Ridge National Laboratory for 1.34 yr. No optical activity was noted, using optical rotary dispersion (ORD) measurements in the region 250–630 nm. At the same time[7] Garay's experiments were extended to include other racemic amino acids, both in the solid state and in neutral, acidic or alkaline solution. In no case, after the same 1.34-yr exposure, did we notice the development of optical activity either by ORD measurements or (in the case of DL-leucine) by the gas-chromatographic determination of the enantiomeric composition of the irradiated samples. Our explanation[7] for these negative results has been that most of the bremsstrahlung causing radiolysis of the samples was of low energy[8] and therefore of very slight circular polarisation[3,9]. In the hope of overcoming this difficulty, we have now undertaken similar experiments using longitudinally polarised electrons (and their bremsstrahlung) from a linear accelerator.

Our source of polarised electrons (located in the W. W. Varian Laboratories at Stanford University; see ref. 10) consists of a rotatable cathode producing a stream of 50–180-V electrons which in turn impinge on and are scattered by a beam of mercury atoms emerging from a heated mercury oven in a vacuum chamber. The scattered electrons are transversely polarised with spin up or spin down. Scattered electrons of the desired transverse polarity are selected by rotating the cathode to obtain appropriate scattering angles, and are then sent through the accelerator section of the apparatus to produce a beam of 120-keV electrons. The degree of polarisation is established by placing a Mott analyser in the beam temporarily, and the transverse polarisation is then converted to longitudinal polarisation by sending the electron beam through a 111° electrostatic beam rotator (that is, producing a relativistic 90° rotation for 120-keV electrons). The emerging longitudinally polarised electrons (natural, antiparallel, 'left-handed', or unnatural, parallel, 'right-handed') then impinge

Table 1 Irradiation of DL-leucine with longitudinally polarised electrons

Polarisation of electrons		Irradiation,	Sample	Composition of sample (corrected*)			Enantiomeric selectivity
Sense	Percentage excess	(nA h)	decomposition (%)	%D	%L	±	(%L − %D)
Antiparallel	~ 20	331	52.9	49.29†	50.71	0.20	1.42
Antiparallel	17–23	498	50.9	49.57‡	50.43	0.14	0.86
Parallel	11–17	795	73.9	50.40§	49.60	0.12	−0.80
Parallel	10–17	860	75.6	50.37¶	49.63	0.14	−0.74

* To analysis of DL-leucine standard.
† Four sample analyses; six DL-leucine standard analyses (±0.22%).
‡ Seven sample analyses; eleven DL-leucine standard analyses (±0.13%).
§ Ten sample analyses; twelve DL-leucine standard analyses (±0.18%).
¶ Thirteen sample analyses; fifteen DL-leucine standard analyses (±0.15%).

on the target substance held in place by Collodion on a thin sheet of aluminium foil backed by a layer of scintillation plastic (to assist focusing) and mounted on a glass window. The beam current of our 120-keV polarised electrons varied from 10 to 35 nA and the degree of polarisation from 10 to 23%.

The target material irradiated was DL-leucine (samples, 15–17 mg, ~ 20 mg cm^{-2} thick). Irradiation times were selected to produce 50–75% degradation of the leucine sample, as determined gas chromatographically by the 'enantiomeric marker' technique[11]. The total dose was approximately 10^9 rad. Asymmetric degradation of the samples was estimated by determining the enantiomeric composition of the undecomposed leucine residue gas chromatographically, converting the irradiated sample to its N-trifluoroacetyl isopropyl ester derivative and analysing on a 150 foot × 0.02 inch capillary column coated with N-lauroyl-L-valyl-*tert*-butylamide phase[12]. All irradiated samples were analysed in replicate, interspersed 'back to back' with similar replicate analyses of non-irradiated DL-leucine derivative as controls. Analyses were carried out with a Hewlett-Packard 5700A Gas Chromatograph coupled to a Hewlett-Packard 3380A Digital Electronic Integrator.

Table 1 indicates the results obtained so far in four separate irradiation experiments, two with longitudinally polarised electrons having the antiparallel left-handed spin characteristic of natural β-decay electrons[13] and two with electrons having unnatural right-handed longitudinal polarisation. We see qualitatively that natural left-handed electrons bring about the degradation of D-leucine more extensively than that of L-leucine on irradiation of racemic DL-leucine, whereas unnatural right-handed electrons engender the asymmetric decomposition of DL-leucine in strictly the opposite sense. Unfortunately, control over the many experimental parameters of our polarised electron source—particularly the beam current and degree of net polarisation—is rather difficult, so that the data in the separate experiments of Table 1 can be compared only in a qualitative or at best merely semiquantitative fashion. It is interesting, however, that the natural left-handed electrons in our experiments produced an asymmetric degradation favouring the formation of an excess of the naturally occurring L-leucine enantiomer

We have evaluated the analytical data of each experiment in Table 1 using the standard statistical *t*-test. Comparison of the product composition percentages in each experiment with the composition data for the simultaneously conducted replicate control analyses with N-TFA-DL-leucine isopropyl ester suggest that the composition data in each separate experiment of Table 1 differ from 50 : 50 at well over the 99.9% confidence level. In spite of such statistical assurances of the validity of our results, however, we have felt it additionally desirable to convince ourselves experimentally that the small differences from 50 : 50 which we observe in the compositions of our irradiated samples were in fact reproducibly detectible and valid using our gas chromatographic technique. Accordingly, known mixtures of D- and L-leucine of compositions approximately 49.0–51.0% and 49.5–50.5%, respectively, were prepared, converted to their N-TFA isopropyl ester derivatives, and analysed identically by gas chromatography, again interspersed 'back to back' with DL-leucine control analyses. The results of these experiments are shown in Table 2; *t*-test evaluation again indicates validity at above than the 99.9% confidence level. We thus have both experimental and statistical justification for tentative faith in the validity of the data on the irradiated samples in Table 1.

Earlier workers have been uniformly unsuccessful in inducing optical activity in racemic organic substrates by irradiations using either a variety of β-ray bremsstrahlung sources[4,5,14] or unpolarised electron beams from various accelerators[14]. In addition, one of us[7] has been unable either to repeat Garay's original type of experiment with alkaline DL-tyrosine or to observe the induction of optical activity in a number of other racemic amino acid substrates as well, in spite of using a ^{90}Sr source some 1.7×10^8 times more powerful than that used by Garay[6]. Accordingly, we believe that our present tentative observations of the asymmetric degradation of a racemic amino acid by longitudinally polarised electrons may represent the first demonstration of the potential validity of the Vester–Ulbricht mechanism for the abiotic origin of optical activity.

At present we do not know whether the asymmetric degradations in Table 1 are caused by the longitudinally polarised electrons themselves, or by their bremsstrahlung.

Table 2 Gas chromatographic analyses of D- and L-leucine mixtures of compositions approximating those of irradiated samples

	DL-leucine standard			Mixture A*			Mixture B*		
	%D	%L	±	%D	%L	±	%D	%L	±
Known	50.00	50.00	–	49.03	50.97	–	49.51	50.49	–
Observed	50.00†‡	50.00	0.08	49.05§	50.95	0.07	49.58¶	50.42	0.09

* Corrected to DL-leucine standard.
† Corrected from 50.24 ± 0.08 observed.
‡ Average of eleven analyses.
§ Average of four analyses.
¶ Average of six analyses.

Relevant experiments are under way, as are attempts to enhance the extent of asymmetric degradation observed in such experiments and to extend them to encompass other amino acid substrates.

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Colour illusion and evidence for interaction between cone mechanisms

IF one adapts the eye for several minutes to a yellow field of 700 cd m^{-2} and then looks at a small blue target, the latter appears green for 2 or 3 s. Target and field can be provided conveniently by Wratten 12 and Illford 622 filters respectively. But the effect fails not only if the adapting field is too dim, but also if it is too bright.

This illusion may be the suprathreshold counterpart of a remarkable, but neglected, observation made in 1949 by Stiles[1]: when a long-wavelength adapting field was turned off, the detection threshold for the blue-sensitive mechanism of the eye increased. We have re-examined this paradoxical loss of sensitivity to determine whether there is evidence here for an inhibitory interaction between different classes of receptor.

The existence of the phenomenon is in doubt: Das[2] reported results similar to those of Stiles, but there is no evidence for the anomaly in the results given by Du Croz and Rushton[3]. We therefore began by measuring the threshold for violet target flashes in the first seconds after a long-wavelength field had been extinguished. A yellow (580 nm) adapting field, which subtended a visual angle of 6.5°, was presented in Maxwellian view For 3 s every 18 s the field was interrupted by a dark interval. At a particular delay after each extinction of the field a 445-nm target flash was introduced. The latter lasted 15 ms, subtended a visual angle of 1° and was delivered to the centre of a fixation array consisting of four white points arranged in a diamond. The test flash and adapting field were concentric. The experiment was under computer control and the threshold for detecting the flash on 50% of trials was measured by a double staircase procedure[4]. No data were gathered until the field had been cycling for 4 min. A block of 50 trials was devoted to the measurement of each threshold and all stimuli within a block were presented at the same delay.

Typical results are shown in Fig. 1, where the log threshold for the 445-nm test flash is plotted at various delays after the yellow field has been turned off. The broken line shows the threshold for the same target when the field is present. Clearly the threshold does not follow a normal dark adaptation curve, but rises immediately after the field has been turned off and after 2.5 s has not yet regained the value it had when the adapting field was present. A target that is readily visible when the background is present becomes invisible when the field is extinguished.

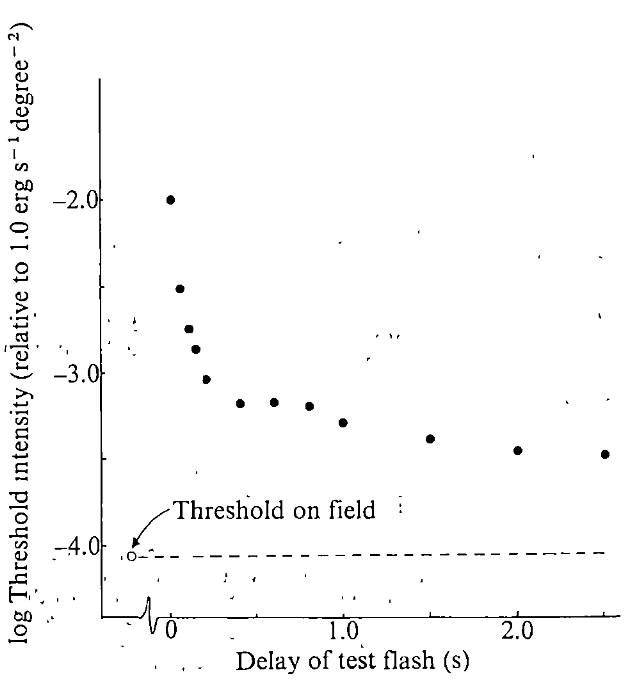

Fig. 1 Log threshold intensity for a violet test flash at various delays after the offset of a yellow field. The intensity of the field was −1.4 log erg s^{-1} deg^{-2}. The broken line shows the threshold when the field is present. Observer, P.G.P.

The suppression of the blue-sensitive mechanism is even greater than at first appears, for we found that the mechanism responsible for detection of violet flashes after the field has been extinguished has the spectral sensitivity of π_4, Stiles' green-sensitive mechanism. The threshold for the blue-sensitive mechanism must lie above the solid points in Fig. 1. Our results so far suggest that the suppression is confined to the blue-sensitive mechanism. Therefore we provisionally call the phenomenon transient tritanopia and suggest that the suprathreshold illusion arises from the change in the relative sensitivity of green and blue cones to short-wavelength light.

In a second experiment a 580-nm field was turned off as before but was replaced immediately by a dimmer green field of 525 nm that had been empirically adjusted to have the same adaptive effect on the blue mechanism in the steady state. The threshold for 445-nm flashes was then measured at various delays after the transition between the two fields. When considering this experiment it is useful to refer to the principle of univariance[5]. A single retinal cone, or a single class of cones, is colour blind. The input to a particular cone can vary in both wavelength and intensity, but once a quantum has been absorbed all information about its wavelength is lost. In principle, therefore, it is possible to adjust the intensities of two adapting fields until they are indistinguishable to the blue cones. According to the classical model of Stiles the adaptive states of the cone mechanisms are independent: that is, the sensitivity of an individual mechanism depends only on the number of quanta absorbed from an adapting field by that particular mechanism; and thus two adapting fields that raise the threshold equally for the short-wavelength target must be producing an equal quantum catch in the blue cones. If the sensitivities of the mechanisms are indeed independent in the steady state and if they remain independent in transitional states then transient tritanopia must

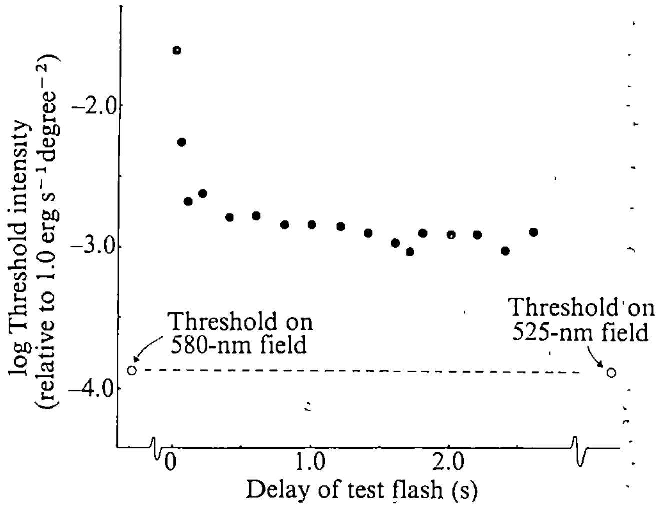

Fig. 2 Solid circles show the threshold for a 445-nm target at various delays after the transition between 580-nm and 525-nm fields. The two fields were adjusted to raise the threshold by the same amount in the steady state: the two open circles, each based on ten determinations, show the empirical thresholds obtained in the steady state. Observer, J.D M.

be a private matter among the blue cones and there should be no disturbance of sensitivity when we switch from the yellow to the green field. If, however, the threshold still rises after the transition, then there must be an interaction between mechanisms: the interaction may occur in the steady state, so that fields that raise the 445-nm threshold equally do not produce equal absorption in the blue cones (such a suggestion has been made, see for example, ref. 6); alternatively or in addition, a recovering long-wavelength mechanism may inhibit, or otherwise suppress, the blue cones during early dark adaptation, for the number of quanta absorbed by the long-wavelength mechanisms will fall as the transition is made between yellow and green fields that have the same adaptive effect on the blue cones.

The threshold for 445-nm flashes was measured on a steady yellow field of −1.36 log erg s^{-1} degree^{-2} and a steady green field was then adjusted until it raised the threshold for the violet flash to the same level. In a final check the thresholds on the two steady adapting fields were repeatedly measured in an alternating sequence. The two mean thresholds, each based on ten determinations, were within 0.01 log$_{10}$ units of each other and were approximately 0 5 log units above the value for the dark-adapted eye. Auxiliary measurements of increment threshold functions showed that detection was mediated by the blue-sensitive receptor mechanism that was termed π_1 by Stiles[7]. Figure 2 shows that transient tritanopia still occurs when the transition is made between two fields that have the same adaptive effect in the steady state: for several seconds after the substitution of the green field the threshold remains almost 1.0 log$_{10}$ units above its value on either of the steady fields.

We conclude that the blue-sensitive receptors are subject to inhibition from a mechanism with a different spectral sensitivity. Whether the interaction is confined to transitional conditions remains to be determined. Stiles distinguished three short-wavelength mechanisms, π_1, π_2, and π_3: it is possible that we see in transient tritanopia an exaggeration of an inhibition that marks the difference between these mechanisms in the steady state.

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Learning in the absence of forebrain noradrenaline

CONSIDERABLE attention has been given to suggestions that forebrain noradrenaline (NA) plays a central role in the associative learning process[1,2]. For example, Kety[2] has proposed a general noradrenergic theory of learning in which he hypothesises that NA release associated with reinforcement facilitates the formation of learned associations. Crow[3] subsequently suggested that the dorsal NA bundle which extensively innervates the cortex could be the crucial pathway involved. In support of this hypothesis are findings that learning on shock avoidance[4] and appetitive[5–7] tasks is impaired after widespread loss of forebrain NA and dopamine. More specifically, Anlezark *et al.*[8] found that bilateral electrolytic lesions to the origins of the dorsal NA bundle in the locus coeruleus impaired the acquisition of runway behaviour. Cortical forebrain NA loss was reduced to 69% of control levels in their study. We have used 6-hydroxydopamine (6-OHDA) treatments to achieve virtually total forebrain NA loss and have found no impairment on the acquisition of runway behaviour. The NA-depleted animals, were however, slow to inhibit running during extinction, suggesting that although the dorsal NA system is not involved in the acquisition of reinforced responses, it is important for the response to non-reward.

Fig. 1 Acquisition of running in alleyway. Subjects were deprived to 90% of free feeding weight. *a*, NPT: *b*, DBT. 6-OHDA was dissolved in 0.9% NaCl containing ascorbic acid (1.0 mg ml^{-1}). Infusion rate was 1 µl min^{-1} in the DBT preparation. Appropriate sham-operated and injected controls were prepared for both groups Time to run from start to goal box plotted against day of training. Time values are means of the five trials on each day. □, Controls; ■, treated.

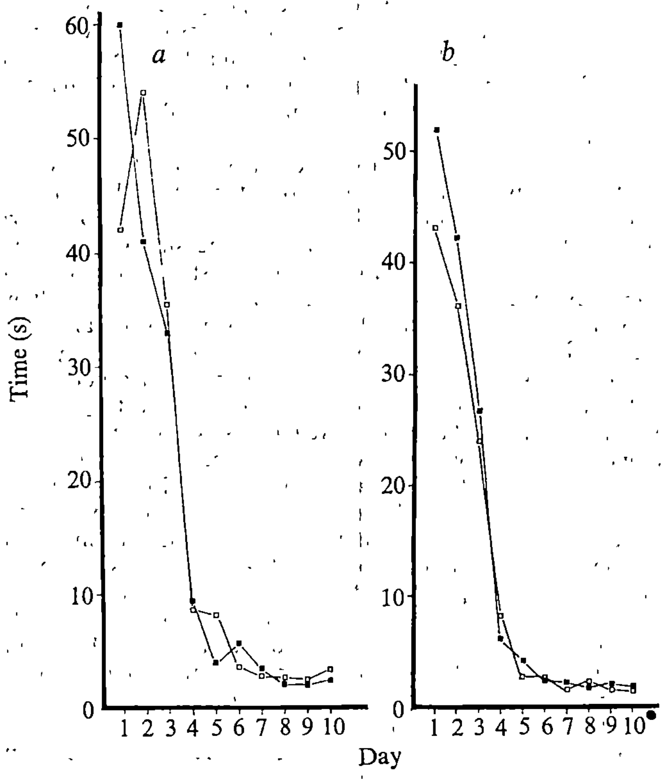

Table 1 Regional concentrations of NA and dopamine (pg per g tissue) in treated and control animals on the two depletion techniques

	NPT Controls ($n = 9$)	NPT Treated ($n = 9$)	*t* Statistic		DBT Controls ($n = 5$)	DBT Treated ($n = 5$)	*t* Statistic	
NA								
Hippocampus	0.415 ± 0.13	0.010 ± 0.007	9.34	$P < 0.001$	0.233 ± 0.069	0.0 ± 0.0	7.58	$P < 0.001$
Hypothalamus	1.10 ± 0.061	1.34 ± 0.094	6.43	$P < 0.001$	0.708 ± 0.365	0.118 ± 0.141	3.38	$P < 0.01$
Cortex	0.205 ± 0.013	0.017 ± 0.002	42.9	$P < 0.001$	0.174 ± 0.068	0.001 ± 0.002	5.71	$P < 0.001$
Rest of brain	0.365 ± 0.040	0.508 ± 0.035	8.07	$P < 0.001$	0.218 ± 0.049	0.208 ± 0.027	0.41	$P > 0.05$
Dopamine								
Hypothalamus	0.43 ± 0.13	0.32 ± 0.09	2.08	$P > 0.05$	0.079 ± 0.167	0.151 ± 0.213	0.60	$P > 0.05$
Striatum	4.71 ± 0.30	4.43 ± 0.32	1.92	$P > 0.05$	5.45 ± 1.82	7.03 ± 2.83	1.05	$P > 0.05$

Values are followed by s. d.

Two different 6-OHDA treatments were used, both of which result in adult rats with virtually total loss of NA from the cortex and hippocampus. In the systemically treated neonate preparation (NPT), 10 albino Wistar rats were injected intraperitoneally on days 1, 3, 5, 7, 9, 11 and 13 after birth with 6-OHDA hydrobromide (100 mg kg^{-1}). Behavioural testing was begun at 3 months of age. In the second technique (DBT), 10 male albino Wistar rats, 2.5 months old received bilateral injections of 6-OHDA into the dorsal bundle using stereotaxic coordinates derived from König and Klippel[9]. Behavioural testing started 2 weeks after the operation. An L-shaped runway was used, of stem length 100 cm and arm length 30 cm; it was painted matt black throughout. The subject was placed in the start box and the door raised when the subject was orientating towards it. The running time in the stem was measured using electronic timers triggered by photocell beam interruption. The beams were located immediately beyond the start box and immediately before the goal box. On entering the goal box the subject was presented with a shallow glass food cup containing four food pellets (45 mg each, P. J. Noyes Company, Lancaster, New Hampshire). At the end of a trial the subject was constrained in the goal box for 10 s; after about 15 s in an inter-trial cage the subject was returned to the start box for the next trial. Five trials a day were carried out until running speeds had reached a plateau (three successive days being within 10% of each other). All animals had reached this plateau within 10 d. Extinction was begun on day 11 and the procedures were identical except that no food cup or food were present in the goal box. Extinction trials continued until the subject's latency was ≥ 10 times the latency on trial 1 of extinction.

No difference in the number of trials to criterion was found between either treated group and its control group during the acquisition period of L-maze running (Fig. 1) (analysis of variance—NPT: $F = 0.058$, d.f. = 1.18, $P \gg 0.05$; DBT: $F = 0.46$, d.f. = 1.18, $P \gg 0.05$). Furthermore, considering speed of running there was no difference between treated and control groups on trial 1 of acquisition (NPT: *t* statistic = 0.29, d.f. = 16, $P \gg 0.05$; DBT: *t* statistic = 1.21, d.f. = 21, $P > 0.05$) confirming that neither of the two NA depletion techniques had produced a nonspecific motor impairment. Both treated and control groups started extinction after the last acquisition trial running at the same speed (trial 1 extinction, NPT, *t* statistic = 1.09, d.f. = 16, $P > 0.05$; DBT: *t* statistic = 0.095, d.f. = 18, $P > 0.05$). On extinction, however, both treated groups showed maintained rapid running and resistance to extinction (Fig. 2) (number of trials to extinction criterion, Mann–Whitney *U* Test—NPT: $U = 3.50$, $P < 0.002$; DBT: $U = 2.00$, $P < 0.001$).

At the end of testing the nine surviving NPT rats and five DBT rats together with their controls were decapitated, and their brains rapidly dissected on ice and subdivided into brain stem, striatum, hypothalamus, cortex and hippocampus. These brain areas were homogenised in perchloric acid and assayed for regional concentrations of NA and dopamine according to Cuello *et al.*[10]. The results confirmed that both treatments had produced severe depletion of NA in the cortex and hippocampus, leaving brain dopamine intact (Table 1).

These results show that with two different selective chemical techniques which result in severe loss of hippocampal and cortical NA, acquisition of L-maze running for food reward was not impaired. This contrasts with the impairment reported[8] to occur after surgical lesions to the locus coeruleus which also deplete forebrain NA, albeit to a lesser degree. We suggest that in the case of surgical lesions the impairment may stem from effects of the lesion other than the observed depletion of NA.

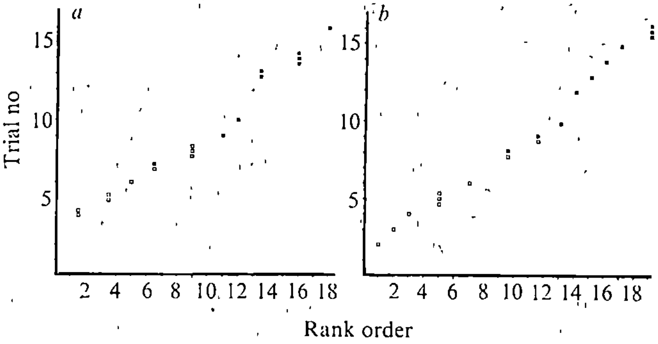

Fig. 2 Extinction of alleyway running. *a*, NPT; *b*, DBT. Trial number on which extinction criterion (see text) was reached plotted against overall rank order of extinction over both groups for each animal. Tied ranks represented by mean value of ranks and with multiple points at that value. □, Controls; ■, treated.

The perseveration in extinction after both treatments reported in the present experiments indicates, however, that forebrain NA depletion produces marked changes in behaviour. The dorsal bundle lesioned animals also show resistance to extinction of bar pressing after training on a continuous reinforcement schedule, and in perseveration to the negative stimulus in a successive light–dark discrimination task (S.T.M. and S.D.I., unpublished). These are all situations in which behaviour is required to change in response to non-reward. Gray[11] has proposed that a particular frequency of θ electrical activity in the hippocampus reflects the coding of non-reward. This frequency (7.7 Hz) has the lowest threshold of elicitation by electrical stimulation of the pacemaker cells for the θ rhythm located in the medial septal area. This threshold is selectively raised by anti-anxiety drugs. The finding of Gray *et al*[12] that lesions of the dorsal NA bundle act in the same way as the anti-anxiety drugs, in selectively raising the threshold for 7.7-Hz septal-induced hippocampal electrical activity, is further support for a role of this NA system in processes concerned with non-reward. Further research to elucidate these effects is planned and it is hoped to develop 6-OHDA lesion techniques which will selectively deplete either cortical or hippocampal noradrenaline.

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Effect of minor tranquillisers on hippocampal θ rhythm mimicked by depletion of forebrain noradrenaline

THERE are similarities between the behavioural effects of minor tranquillising drugs and those of lesions to the septal area and hippocampus[1]. Since the septal area contains the pacemaker cells for the hippocampal theta rhythm[2], a parsimonious hypothesis to account for these common effects is that tranquillisers affect behaviour by altering septal control of this rhythm. Sodium amylobarbitone (SA) has been shown to block the behavioural effects of frustrative non-reward[1], as do septal[3] and hippocampal[4] lesions. Rats exposed to non-reward in the runway show a characteristic hippocampal θ response at 7.7 Hz (ref. 1) (the θ frequency range in the rat is about 6–12 Hz). Higher and lower frequencies occur during forms of behaviour which are unaffected by the drug. It has therefore been proposed[1] that the behavioural effects of SA arise by virtue of an impairment of septal control of the hippocampal θ rhythm specifically in a frequency band centred on 7.7 Hz. In support of this, it has been demonstrated[5] that this drug raises the threshold for septal driving of the hippocampal θ rhythm selectively at 7.7 Hz, frequencies both above and below this value being minimally affected. Here we show that this kind of frequency-specific effect on septal driving of the hippocampal θ rhythm is also produced by other drugs with similar effects on behaviour[1] (ethanol, chlordiazepoxide and Δ^9-tetrahydrocannabinol (Δ^9-THC)), by drugs which impair noradrenergic neural transmission, and by destruction of the dorsal ascending noradrenergic bundle, by intracerebral injection of 6-hydroxydopamine (6-OHDA)[6].

The techniques used for electrode implantation and for stimulation and recording have been described previously[1,7]. The subjects were free-moving male albino rats with a bipolar stimulating electrode chronically implanted in the medial septal nucleus and a bipolar recording electrode in the dorso-medial subiculum. (J. N. P. Rawlins has shown that the θ rhythm recorded here is identical in phase and frequency to that recorded in CA1.) In this preparation it is possible to drive the hippocampal theta rhythm by septal stimulation, the frequency of the driven wave corresponding exactly to the frequency of the applied stimulation. To obtain a driving threshold at a particular frequency of septal stimulation, a Tektronix 502A dual-beam oscilloscope was set to be triggered by the stimulus pulse (0.5 ms d.c., square wave, from an isolated constant-current source) and the stimulating current was gradually increased in intensity until the hippocampal EEG (displayed on the second beam of the oscilloscope after amplification by a Grass 7P5B pre-amplifier) showed waves which remained in phase with the stimulus. Five stimulating frequencies were used (see Fig. 1) and five readings were taken in random order at each of these frequencies over a 30-min experimental session. The readings were then averaged to construct a 'θ-driving' curve (Fig. 1). The stability of the readings obtained in this way is good, as is agreement between different experimenters. In the undrugged male rat there is a minimum or dip located at 7.7 Hz (Fig. 1). In a control series of 26 male rates, 24 (92%) displayed such a dip.

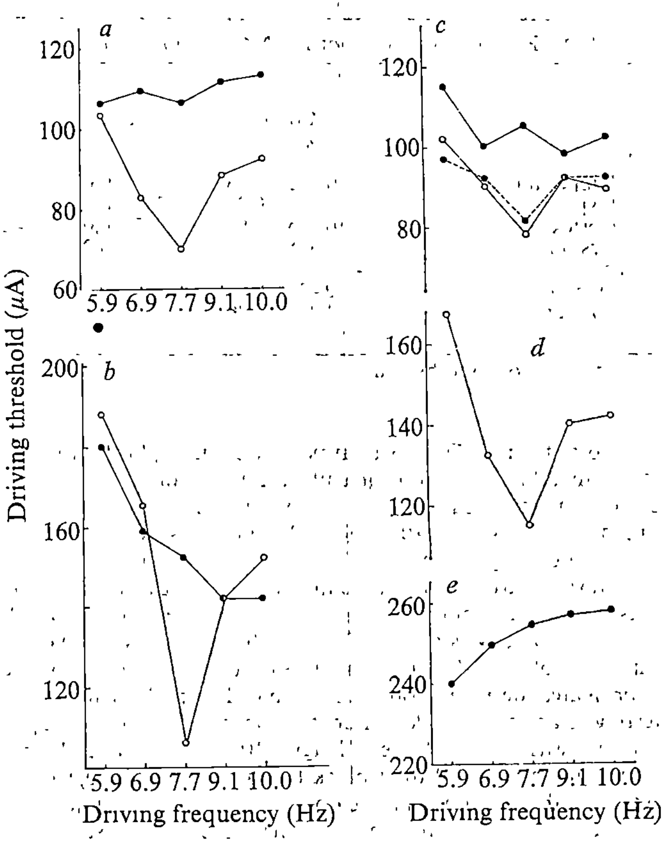

Fig. 1 Effects of various treatments on threshold for septal driving of hippocampal θ rhythm as a function of stimulation frequency (θ-driving curve). *a*, *b*, and *c*, Average results obtained from a group of male rats before and after drug treatment. Control injections consisted of appropriate vehicle alone. *a*, Chlordiazepoxide HCl, $n=4$, 5 mg kg^{-1} intraperitoneally (i.p.) in 1 ml kg^{-1} saline 30 min before test. *b*, Δ^9-THC, $n=4$, 0.5 mg kg^{-1} i.p. in 1 ml kg^{-1} Tween 80–saline solution 20 min before test. *c*, α-MPT, $n=4$, 100 mg kg^{-1} i.p. in 1 ml kg^{-1} saline followed 7.25 h later by 30 mg kg^{-1} *dl*-threo-3,4-dihydroxyphenylserine (DOPS) i.p. suspended in 1 ml kg^{-1} saline; readings were taken 6.5 h after α-MPT and 70 min after DOPS. *d*, Average of three rats given control injection of saline–ascorbic acid vehicle in dorsal ascending noradrenergic bundle; *e*, average of three rats injected with 6-OHDA in dorsal bundle. *a* and *b*, ●, Drug; ○, control: *c*, ●, α-MPT; - - -, DOPS; ○, control.

We have investigated, in a total of 19 rats, the changes produced in the θ-driving curve by injections of SA, ethanol, chlordiazepoxide and Δ^9-THC. The drug parameters used are known to be behaviourally effective in relevant situations[1,8]. All four produced their largest effect at 7.7 Hz, thus eliminating the dip in the curve at this frequency. The results with chlordiazepoxide and Δ^9-THC are illustrated in Fig. 1*a* and *b*. The results with 15 mg kg^{-1} SA and 1,200 mg kg^{-1} ethanol closely resembled those obtained with the other two drugs, as well as those previously obtained with 20 mg kg^{-1} SA (ref. 5). The data from the four drug treatments were logarithmically transformed (to eliminate inhomogeneity of variance) and submitted jointly to analyses of variance for the effects of drug

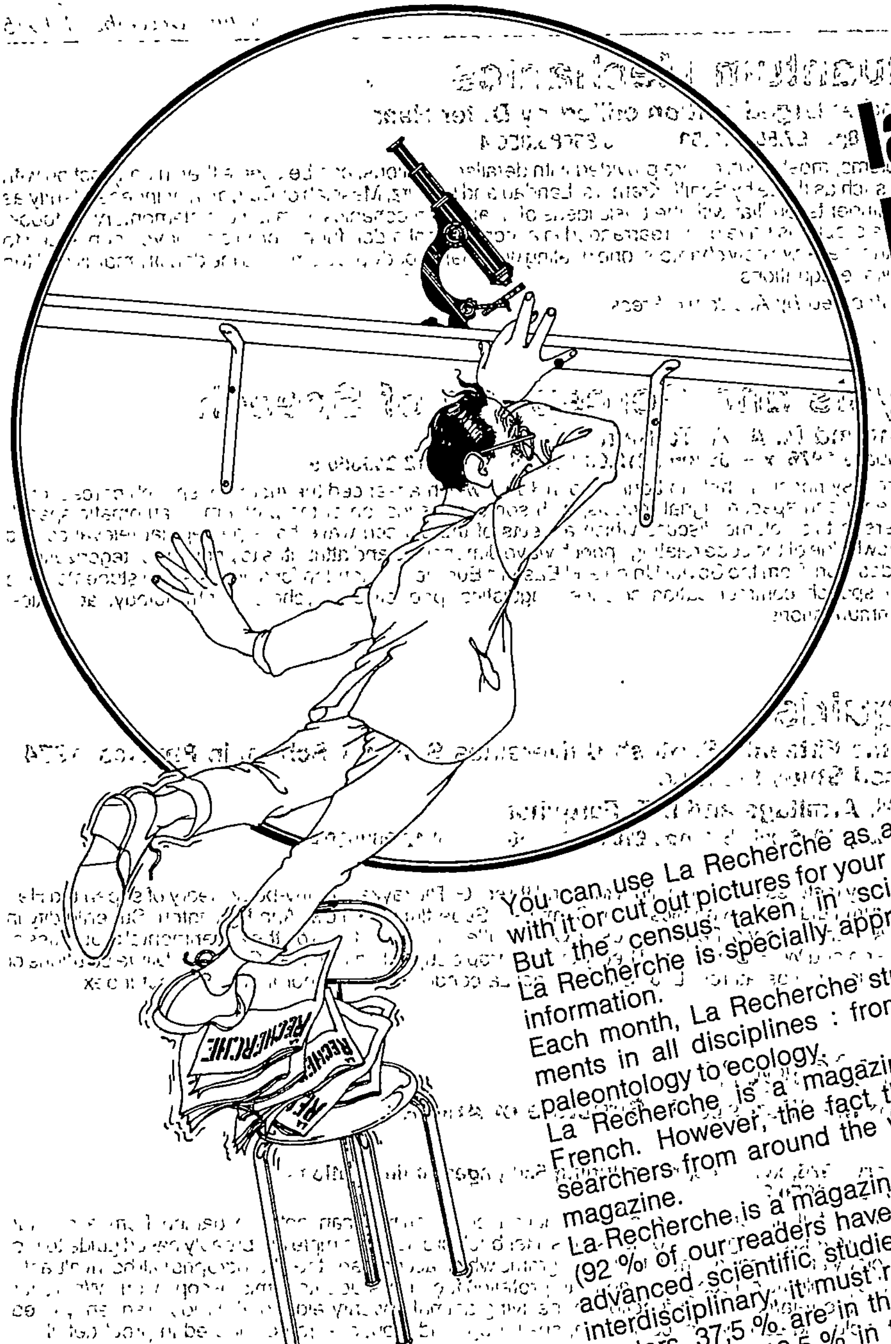

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Table 1 Effect of 6-hydroxydopamine injection into dorsal ascending noradrenergic bundle on regional levels of noradrenaline and dopamine

	Vehicle injected ($\mu g\ g^{-1}$)	6-OHDA injected ($\mu g\ g^{-1}$)	t	P
Hippocampus NA	0.30 ± 0.04	0.01 ± 0.01	9.67	<0.001
Hypothalamus NA	1.97 ± 0.75	0.97 ± 0.28	1.71	NS
Hypothalamus DA	0.54 ± 0.13	0.53 ± 0.09	0.06	NS
Striatum DA	10.22 ± 3.07	8.49 ± 1.13	0.75	NS
Cortex NA	0.39 ± 0.16	0.04 ± 0.03	3.05	<0.025
Septum NA	0.82 ± 0.34	0.31 ± 0.12	1.99	NS

Values shown are means ($n = 3$) ± standard error.
NS, Not significant.

(that is, the four separate drugs), treatment (that is, drug against placebo) and stimulation frequency. There was a significant interaction between treatment and stimulation frequency ($F = 4.4$; d.f. $= 4$ and 135; $P < 0.01$) but the drug × treatment × frequency interaction was small ($F < 1$), both tested against the within-subject residual variance. Thus, the four drugs had essentially the same frequency-specific effect: the threshold at 7.7 Hz was significantly lower than at any other frequency in the combined control condition but not after treatment with any of the drugs.

We have tried to mimic the characteristic change produced in the θ-driving curve by the minor tranquillisers by using other drugs with better understood neurochemical effects. We have been unable to do this with cholinergic or anticholinergic drugs (0.05 or 0.5 mg kg^{-1} physostigmine, 0.5 mg kg^{-1} scopolamine); and Stewart and Gray (unpublished) failed to do so by blocking the synthesis of 5-hydroxytryptamine (with 300 mg kg^{-1} *p*-chlorophenylalanine). We have, however, obtained evidence of a function for noradrenergic neurones. We used α-methyl-*p*-tyrosine (α-MPT), which blocks the synthesis of dopamine (DA) and noradrenaline (NA)[9], followed by dihydroxyphenylserine (DOPS), an artificial precursor of NA but not of DA[10]. α-MPT acted on the θ-driving curve as did the tranquillisers and this action was reversed by DOPS (Fig. 1*c*). Analyses of variance showed a significant treatment × frequency interaction ($F = 6.3$; d.f. $= 4$ and 12; $P < 0.01$) for α-MPT against control, but no significant difference from control after further treatment with DOPS ($F < 1$).

These results indicate a noradrenergic pathway which facilitates septal driving of the hippocampal θ rhythm maximally at a frequency of 7.7 Hz. The pathway most likely to produce this effect is the dorsal ascending noradrenergic bundle originating in the locus coeruleus, which innervates the neocortex, medial septal area and hippocampus[6,11]. We used intracranial injections of 6-OHDA to destroy this pathway. Eight rats were anaesthetised and placed in a stereotaxic instrument. A needle, aimed at the dorsal bundle (Plate I of ref. 6), was lowered and 4 or 8 μg of 6-OHDA in 4 μg μl^{-1} of cooled saline containing ascorbic acid (1 μg μl^{-1}) was injected bilaterally at a rate of 1 μl min^{-1}. Bipolar subicular recording electrodes and a septal stimulating electrode were then implanted bilaterally. Of the eight rats so treated, three showed θ-driving (about our usual success rate in unlesioned rats). None displayed a 7.7-Hz dip in the θ-driving curve from either hemisphere (Fig. 1*e*). Comparison with the untreated control series shows this result to be highly significant ($\chi^2 = 16.2$, d.f. $= 1$, $P < 0.001$). In three control rats, given an injection of the saline–ascorbic acid vehicle in the dorsal bundle, the θ-driving curve was normal (Fig. 1*d*).

Regional levels of NA and DA were determined in the 6-OHDA- and vehicle-injected rats. The rats were stunned and decapitated. The brains were dissected on an ice-cold glass plate[12], and NA and DA were assayed by a radioenzymatic method[13]. The results (Table 1) showed that the lesion produced a large (97%) decrease in hippocampal NA, a smaller (non-significant) decrease in hypothalamic NA and no change in hypothalamic or striatal DA. Thus the change produced in the θ-driving curves of the experimental animals probably reflects the loss of the noradrenergic afferents to the hippocampus and/or septal area from the locus coeruleus.

The results of these experiments suggest that the dorsal noradrenergic bundle exercises a frequency-specific facilitation of the septally-controlled hippocampal θ rhythm, and that the minor tranquillisers impair this noradrenergic input to the hippocampus (as previously indicated by biochemical observations[14]). This impairment may, in part, underlie the behavioural effects of the minor tranquillisers. If so, destruction of the dorsal bundle should produce the same effects on behaviour as injection of a minor tranquilliser. Such effects have been found in situations involving frustrative non-reward by Mason and Iversen[15].

We thank the Medical Research Council for support and Dr L. L. Iversen for the use of biochemical facilities. P.H.K. is supported by an ICI postdoctoral Fellowship. The data on Δ^9-THC were kindly provided by A. Drewnowski.

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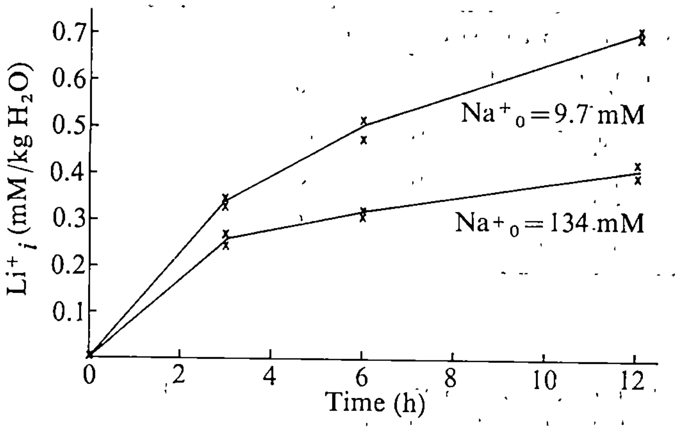
Coupling of lithium to sodium transport in human red cells

THE administration of lithium salts is often used in the treatment of affective psychoses[1]. Mendels *et al.*[2–4] and others[5] have measured the concentrations of lithium in blood plasma and red cells of patients and normal individuals receiving such salts. They have argued that the lithium concentrations in red cells can be correlated with the clinical status of these people[4] and, therefore, can possibly be related to the pathogenesis of the mental disorders. They found that the concentration of lithium in red cell water is about one-third of that in the blood plasma during chronic administration of LiCl (ref. 4). This fact is surprising since it would suggest that Li^+ is actively transported by the red cell membrane. The steady-state value of the ratio



Change in cell composition during experiment

Time	Na^+_0	Li^+_0	H_2O_i	Li^+_i	Na^+_i	K^+_i
(h)	(mM)	(mM)	(w/w)	mM per l original cells		
0	134	1.5	0.658	0.00	7.2	75
3	134	1.5		0.17	15	73
6	134	1.5		0.22	17	69
12	134	1.5	0.642	0.27	19	69
0	9.8	1.5		0.00	7.2	75
3	9.8	1.5		0.22	7.2	76
6	9.8	1.5		0.32	6.4	76
12	9.8	1.5	0.642	0.46	6.6	72

Fig. 1 Typical results from one (experiment 86) of three identical experiments each carried out in duplicate. Fresh human red cells, washed in isotonic NaCl, and incubated (5% v/v) for 12 h at 37 °C in a medium containing LiCl (1.5 mM), KCl (5 mM), glycyl glycine (25 mosM, *p*H 7.3), glucose (5 mM), adenosine (5 mM), ouabain (10^{-4} M) and either NaCl (140 mM) (high Na^+ medium) or NaCl (10 mM) plus $MgCl_2$ (85 mM) (low Na^+ medium). There was no significant change in the composition of the medium during the incubation.

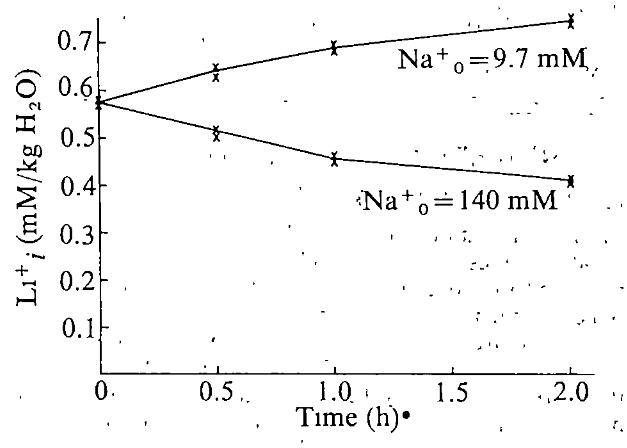
of internal to external concentration for a monovalent cation which is not actively transported should be about 1.2 (ref. 6). Previous observations in red cells[7] and squid axons[8] suggested that Li^+ is not transported by the Na–K pump. Furthermore, the net efflux of Li^+ into Li^+-free medium was unaffected by the presence of ouabain[4]. The experiments described here were designed to clarify the mechanisms of this seemingly ouabain-insensitive 'active' transport of lithium ions in human red cells.

The steady-state concentration ratio for Li^+ is about twice as high in low potassium (LK) as in high potassium (HK) sheep red cells[4], and this is consistent with the hypothesis that Li^+ transport in this system occurs by an ouabain-insensitive process of Li^+–Na^+ exchange diffusion[6]. By this process, Li^+ would move through the red cell membrane only in exchange for some other cation which might be Li^+ or Na^+. If this is the only, or the major, mechanism of Li^+ transport, the steady-state distributions of Li^+ and Na^+ would be similar. If Na^+ is also transported by the ouabain-sensitive Na^+–K^+ pump and the ouabain-insensitive but voltage-dependent leak pathways which maintain the electrochemical potential of Na^+ lower inside than outside of the red cells, then the Na^+–Li^+ exchange process could maintain a similar asymmetry in the distribution of Li^+. In this system, the energy for the 'active' transport of Li^+ would be provided by dissipation of the electrochemical potential difference for Na^+. Our results are consistent with this hypothesis.

We measured the effect of external Na^+ on the uptake and extrusion of Li^+ by red cells freshly drawn from normal human donors. The cells were washed free of plasma with isotonic NaCl, suspended in an appropriate medium (5% v/v) and incubated (37 °C, *p*H 7.4). All media contained LiCl (~ 1.5 mM), KCl (5 mM), glycyl glycine (25 mosM, *p*H 7.4) and ouabain (10^{-4} M). Samples of these cell suspensions taken at intervals were centrifuged, the cells washed in ice-cold, isotonic $MgCl_2$ solutions and lysed[9]. The lysates as well as samples of the extracellular medium, were analysed for Na^+, K^+ and Li^+ by atomic absorption spectrophotometry, and for haemoglobin by spectrophotometry[9]. Water content of the cells was estimated by measuring wet and dry weights.

The results of the first type of experiment are shown in Fig. 1. Cells were incubated in media containing either ~ 140 mM NaCl (high Na^+ medium) or ~ 10 mM NaCl (low Na^+ medium, $MgCl_2$ replaced NaCl to maintain isomoticity). The rate of Li^+ accumulation was substantially greater in cells exposed to the low Na^+ medium. External Na^+ inhibits Li^+ uptake.

Figure 2 describes the results of the second type of experiment. Cells were incubated for 12 h in a low Na^+ medium containing 1.5 mM LiCl. These Li^+-loaded cells were then resuspended in either high or low Na^+ medium. The cells exposed to the high Na^+ medium promptly extruded Li^+ against the electrochemical potential gradient, whereas the cells in the low Na^+ medium



Change in cell composition during experiment

Time	Na^+_0	Li^+_0	H_2O_i	Li^+	Na^+_i	K^+_i
(h)	(mM)	(mM)	(w/w)	mM per l original cells		
0	140	1.6	0.649	0.37	6.4	91
0.5	140	1.6		0.32	9.2	91
1.0	140	1.6		0.29	9.4	89
2.0	140	1.6	0.629	0.26	9.6	86
0	9.7	1.2	0.649	0.37	6.4	91
0.5	9.7	1.2		0.41	6.4	92
1.0	9 7	1.2		0.47	6.5	90
2.0	9.7	1.2	0 641	0.48	6.4	89

Fig. 2 Typical results from one (experiment 90) of three identical experiments, each performed in duplicate. Fresh human red cells washed in isotonic NaCl and incubated (5% v/v) for 12 h at 37 °C in low Na^+ medium were resuspended in either low Na^+ or high Na^+ medium (as defined in Fig. 1) and again incubated at 37 °C. The composition of the medium did not change with time during this second incubation, while the composition of the cells changed as shown in the accompanying table.

continued to gain Li^+. Li^+ is 'actively' transported out of the cells in the presence of an electrochemical potential difference and for Na^+ and ouabain.

Table 1 shows the result of an experiment in which human red cells were loaded with Na^+ and Li^+ by the Nystatin method. When these cells were incubated in media identical to those described in Fig. 1 (except that Li^+_0 was 1.0 instead of 1.5 mM), Li^+ accumulation against an electrochemical potential gradient occurred in cells exposed to a low Na^+ medium but not to a high Na^+ medium. Net uphill transport of Li^+ occurs in either direction across the human red cell membranes depending on the direction of the Na^+ electrochemical potential gradient.

Table 1 Incubation of human red cells

Time (h)	Na^+_0 (mM)	Li^+_0 (mM)	H_2O_i (w/w)	Li_i mM per l original cells	Na_i mM per l original cells
0			0.631	0 91	112
0.5				0.86	106
1.0				0.85	104
2.0	130	1.06	0.637	0.85	112
0			0.631	0.91	112
0.5				1.02	97
1.0				1.22	94
2.0	10	1.02	0.644	1.30	92

These results agree with the hypothesis that ion exchange counter flow is the major mechanism of net uphill Li^+ transport in human red cells. Further confirmation will require demonstration of net uphill Na^+ transport driven by an oppositely-directed Li^+ electrochemical potential gradient. The data support the conclusion that an electrochemical potential gradient for Na^+ is a sufficient condition for such 'active' transport of Li^+. The properties and physiological significance of this Na^+–Li^+ transport system can now be studied. The dependence of the exchange process on external and internal concentrations of Na^+ and Li^+ must be defined. Another line of investigation will be to explore the relationship between the Na^+–Li^+ exchange system and the Na^+–Ca^{2+} exchange system known to operate in nerve membranes[10]. Such a relation could be important in the therapeutic action of Li^+ in view of the known function of Ca^{2+} in the release of neurotransmitters from synaptic vesicles[11]. The existence of ion exchange systems when one partner (for example, Na^+) is also actively transported permits the coupling of the other partner (for example, Li^+) to the active transport process even though the latter is not itself pumped.

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Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity

ATAXIA telangiectasia (AT) is an autosomal recessive defect in man showing among the clinical features[1]: cerebellar ataxia, telangiectasia, IgA deficiency, an enhanced frequency of malignancy and an enhanced level of spontaneous chromosome instability[2]. There have also been reports of increased sensitivity to X rays after radiotherapy[3–5] and increased chromosome aberrations induced by ionising radiation in leukocyte cultures from AT patients[6,7]. We

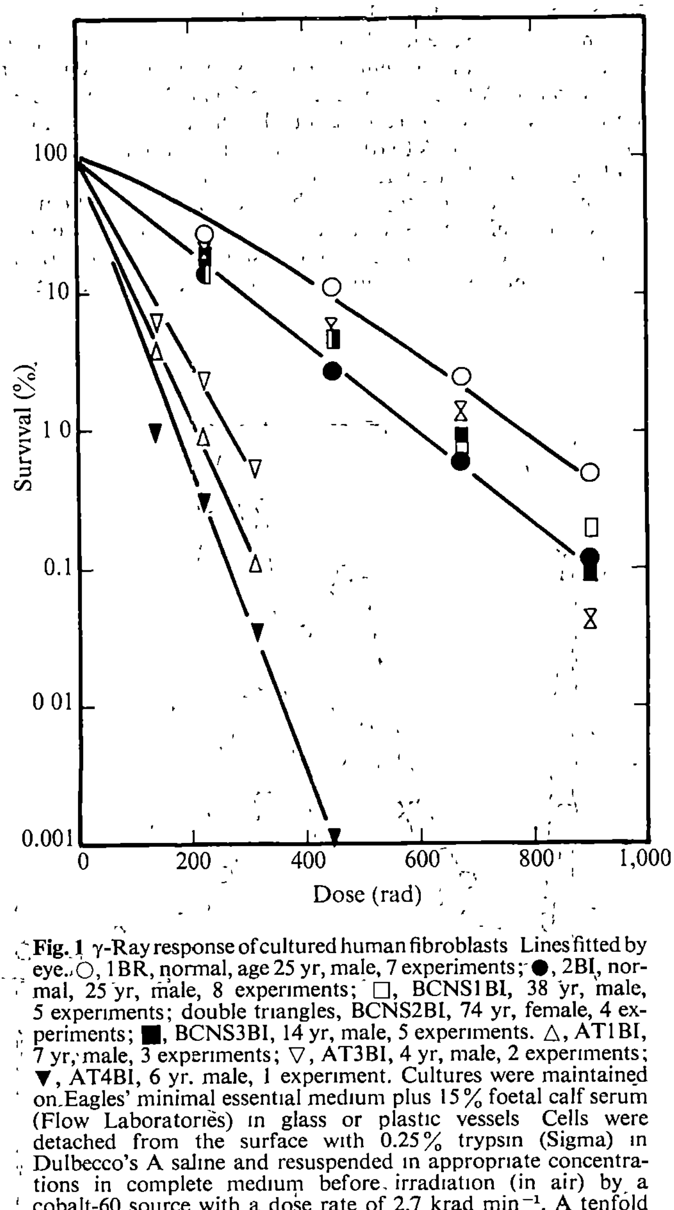

Fig. 1 γ-Ray response of cultured human fibroblasts Lines fitted by eye. ○, 1BR, normal, age 25 yr, male, 7 experiments; ●, 2BI, normal, 25 yr, male, 8 experiments; □, BCNS1BI, 38 yr, male, 5 experiments; double triangles, BCNS2BI, 74 yr, female, 4 experiments; ■, BCNS3BI, 14 yr, male, 5 experiments. △, AT1BI, 7 yr, male, 3 experiments; ▽, AT3BI, 4 yr, male, 2 experiments; ▼, AT4BI, 6 yr. male, 1 experiment. Cultures were maintained on Eagles' minimal essential medium plus 15% foetal calf serum (Flow Laboratories) in glass or plastic vessels Cells were detached from the surface with 0.25% trypsin (Sigma) in Dulbecco's A saline and resuspended in appropriate concentrations in complete medium before irradiation (in air) by a cobalt-60 source with a dose rate of 2.7 krad min^{-1}. A tenfold dilution in unirradiated medium containing 15% foetal calf serum (Sera Services) or calf serum (Flow Laboratories) to remove any influence of radiation on the components of the medium always preceded plating on 9-cm (Nunclon) dishes. Two plating protocols have been practised. (1) The thin feeder layer technique[13]: cultures were kept in sealed polystyrene boxes with a 5% CO_2 in air mixture in an anhydric incubator for 14–16 d and with a medium change after 7 d. (2) Cells were plated without feeder layer and kept in CO_2 incubators with medium changes every 3 d for 14–16 d. At the end of the growth period colonies were stained with 1% methylene blue. A colony containing 50 cells was counted as a survivor. The mean cloning efficiency in this series of experiments was 28% While we have noted a correlation between cloning efficiency and number of cell doublings in a culture, particularly in early subcultures, there was no indication of a correlation between cloning efficiency and cell survival.

report here cell survival experiments which indicate that the clinically observed enhanced sensitivity of AT patients to ionising radiation is manifest at the cellular level.

Figure 1 illustrates the response to γ rays of fibroblast cell lines from the skin of two normal donors, three AT patients and three patients with the basal cell naevus syndrome (BCNS). BCNS has, like AT, shown indications of an association between this condition and enhanced spontaneous and X-ray-induced chromosome aberrations[8]. The response of the normal cell strains falls, in our experience, within the range shown by the two individuals whose survival curves are shown in Fig. 1. (So far we have examined cell strains from eight individuals.) Clearly the BCNS cells fall within the normal range. The cells from the three AT patients, however, show greatly enhanced sensitivity. A similarly enhanced sensitivity was seen after irradiation of AT4BI fibroblasts with 300 keV X rays, when compared with two further control cell strains. It is interesting that patient AT1BI was the same patient in whom Cunliffe *et al.*[5] showed radiosensitivity *in vivo*.

In preliminary attempts to characterise the biochemical basis of the enhanced sensitivity we have compared the production and repair of γ-ray induced single and double-strand breaks in the DNA of normal and AT cells. Like other mammalian cells, human fibroblasts can rejoin single-strand breaks, most being rejoined within 1 h of the post-irradiation incubation, as demonstrated by sedimentation patterns obtained from alkaline sucrose gradients (ref. 9 and confirmed in our experiments). The rates of rejoining of single-strand breaks were very similar in fibroblasts from a normal donor and from an AT patient (Fig. 2*a* and *b*).

Using neutral sucrose gradients, we found in earlier investigations that DNA from unirradiated mouse cells formed a rapidly sedimenting peak (RSP). Ionising radiation released free DNA molecules from the RSP and higher doses of radiation decreased the molecular weight of the free DNA[10]. We suggested that these changes in sedimentation behaviour in non-denaturing conditions might be a consequence of the introduction of double-strand breaks into the DNA. Using similar experimental conditions we have now observed comparable behaviour with human fibroblasts. A dose of 30 krad released most of the DNA from the RSP. During post-irradiation incubation the RSP was reformed (Fig. 2*c*). If the release of DNA from the RSP is indeed a consequence of the introduction of double-strand breaks, then reformation of the RSP, which represents a return to the sedimentation behaviour characteristic of that from unirradiated cells, is probably a manifestation

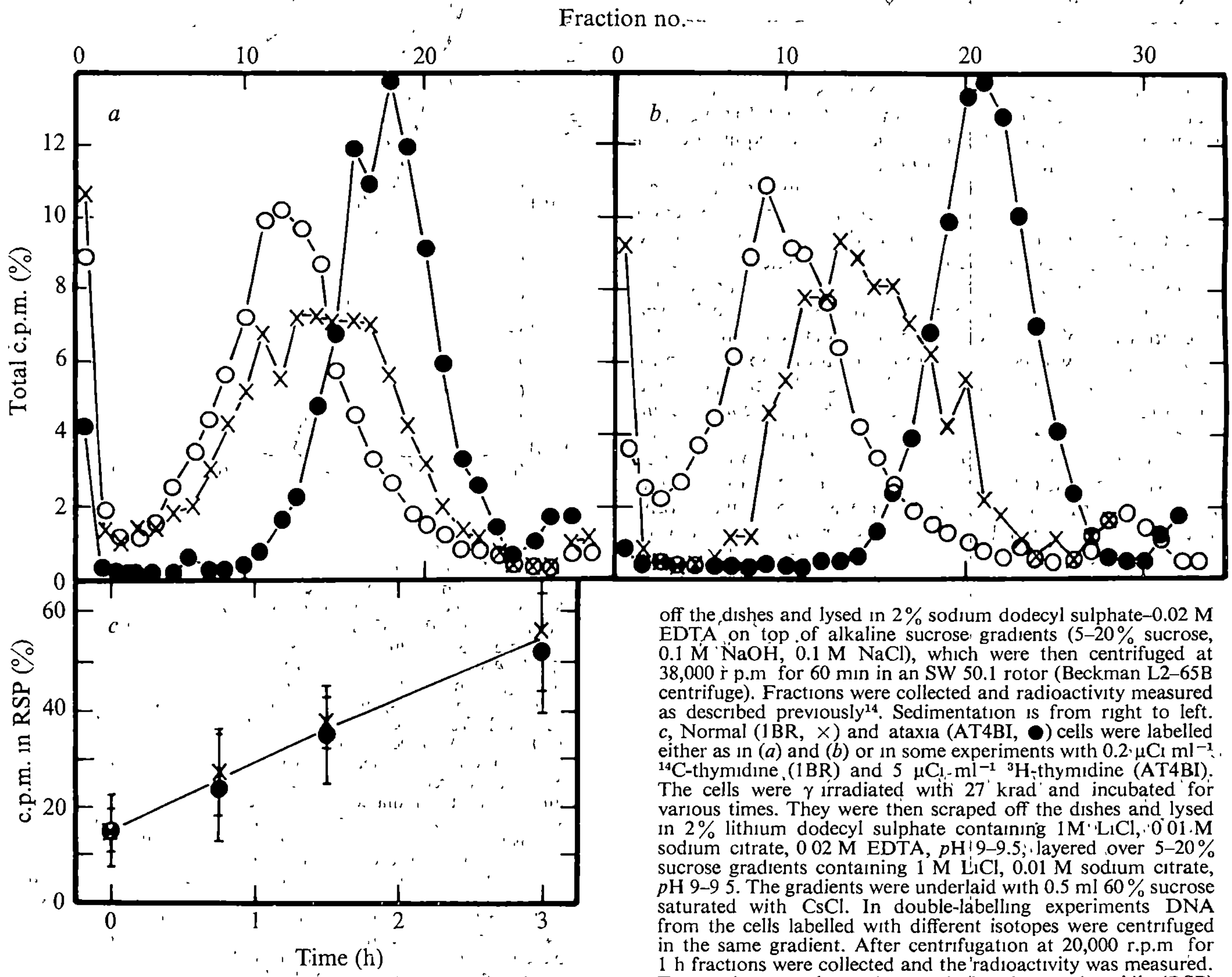

Fig. 2 Rejoining of strand breaks in normal and ataxia cells 10^5 cells of 2BI (*a*) or AT1BI (*b*) were incubated in 5-cm Petri dishes. On the next day the cells were labelled with 1.5–5 μCi ml^{-1} ^{3}H-thymidine (5 Ci mmol^{-1}), for 16–24 h. After removal of medium, the cells were γ irradiated with a dose of 16 krad, and subsequently incubated in fresh medium at 37 °C. After incubation times of 0 (●), 20 (×) or 40 (○) min the cells were scraped off the dishes and lysed in 2% sodium dodecyl sulphate–0.02 M EDTA on top of alkaline sucrose gradients (5–20% sucrose, 0.1 M NaOH, 0.1 M NaCl), which were then centrifuged at 38,000 r.p.m for 60 min in an SW 50.1 rotor (Beckman L2–65B centrifuge). Fractions were collected and radioactivity measured as described previously[14]. Sedimentation is from right to left. *c*, Normal (1BR, ×) and ataxia (AT4BI, ●) cells were labelled either as in (*a*) and (*b*) or in some experiments with 0.2 μCi ml^{-1} ^{14}C-thymidine (1BR) and 5 μCi ml^{-1} ^{3}H-thymidine (AT4BI). The cells were γ irradiated with 27 krad and incubated for various times. They were then scraped off the dishes and lysed in 2% lithium dodecyl sulphate containing 1 M LiCl, 0.01 M sodium citrate, 0.02 M EDTA, *p*H 9–9.5, layered over 5–20% sucrose gradients containing 1 M LiCl, 0.01 M sodium citrate, *p*H 9–9.5. The gradients were underlaid with 0.5 ml 60% sucrose saturated with CsCl. In double-labelling experiments DNA from the cells labelled with different isotopes were centrifuged in the same gradient. After centrifugation at 20,000 r.p.m for 1 h fractions were collected and the radioactivity was measured. Two peaks were obtained, one which sedimented rapidly (RSP) and was held on the sucrose/CsCl shelf, the other being a slower sedimenting free DNA peak. The proportion of the total radioactivity sedimenting in the RSP is plotted against the time of post-irradiation incubation at 37 °C. Results shown are the mean of four experiments. Variation between experiments was much greater than the difference between the two cell lines in any one experiment.

of the rejoining of double-strand breaks. On this basis, we can infer that human fibroblasts, like Chinese hamster cells[11], can rejoin double-strand breaks. Preliminary findings of our experiments (which will be reported in detail elsewhere) indicate that the rate of reformation of the RSP (rejoining of double-stranded breaks) is similar in normal and AT cell strains (Fig. 2*c*)

The human ultraviolet-light sensitive disorder xeroderma pigmentosum has been of great value in elucidating the relationship between ultraviolet sensitivity, defective repair of ultraviolet-induced damage to DNA, enhanced frequency of malignancy and increased levels of cellular mutagenicity[12] The AT syndrome provides an instance where clinical manifestations of damage by ionising radiation[3–5] is reflected at the cellular level. Our preliminary studies on rejoining of DNA strand breaks have not revealed any deficiency in these processes in AT cells. Involvement of DNA damage in the AT syndrome is, however, suggested by the fact that AT cells are also more sensitive than normal cells to actinomycin D, mitomycin C and methyl methanesulphonate (personal communication from D. I Hoar and P. Sargent), and to the chromosome-breaking effects of ionising radiation[6,7]. It is hoped that studies with AT cells will promote a better understanding of the molecular basis of the response of cells to ionising radiation and other mutagens.

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Mutation induction and inactivation in mammalian cells exposed to ionising radiation

MAMMALIAN cell cultures are used to estimate mutation induction by radiation and chemical agents in the belief that they will provide relevant data for assessing risk to man and insights into the mechanisms of mutagenesis in eukaryotic cells It might be anticipated that these estimates would be obtained with the precision characteristic of studies with microorganisms, but very large variations in the response of mammalian cells to ionising radiation have been reported, even by workers using the same cell line and method of mutant estimation[1,2]. Also there has been no unequivocal demonstration of radiation-induced mutation in freshly isolated diploid (homonuclear) cell cultures[3,4]. We consider that these discrepancies arise primarily from methodological difficulties, and report here the similarity of mutation frequency induced by ionising radiation in an established (heteronuclear) cell line used in many previous studies (Chinese hamster V79) and in human diploid cells (early passage foetal lung cells). The mutational response of the two cell types can be considered as identical when the differences in sensitivity to the inactivating effects of radiation are taken into account.

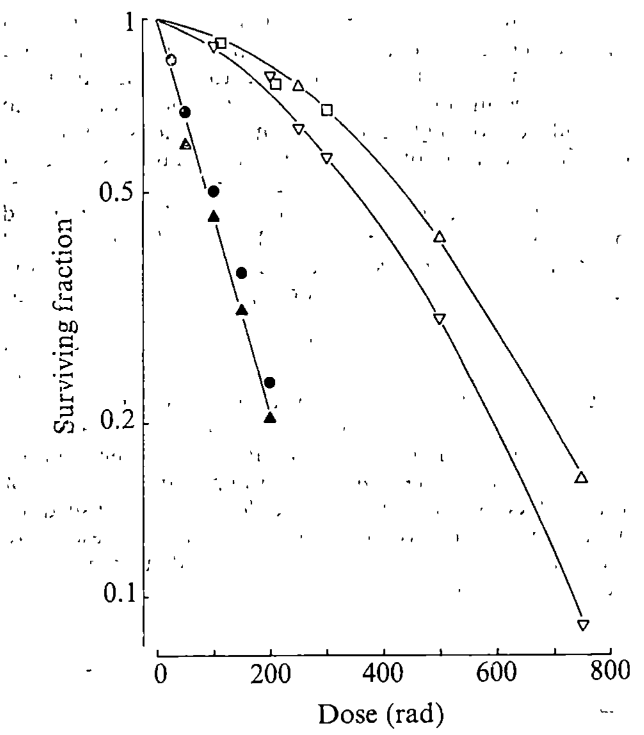

Fig. 1 Survival curves of irradiated human diploid and V79 Chinese hamster cells. Closed symbols, human cells, open symbols, hamster cells. Radiations: △ ▽ ▲ ^{60}Co γ rays; ● 250 keV X rays; □ 5 keV μm^{-1} protons. HF19 human diploid fibroblasts were initiated from female embryonic lung tissue and maintained in monolayer culture[9]. The stock of V79 Chinese hamster cells was originally obtained from Dr E. H Y. Chu; it was held at liquid nitrogen temperature and cells were removed and grown in monolayer culture for several days before an experiment. Both cell types were grown in Eagles MEM plus 10% foetal calf serum (Gibco-Biocult) and exponentially-growing populations were irradiated on plastic Petri dishes at the following dose rates: γ rays, 120 rad min^{-1}; X rays, 50–250 rad min^{-1}; protons, 70–160 rad min^{-1}. Hamster cells were also irradiated as suspensions in growth medium, made up immediately after trypsinising monolayers of cells (▽). After irradiation cells were incubated on plastic Petri dishes for 7 d (hamster cells) or 14 d (human cells) in a gas phase of 5% CO_2 in air at 37 °C Clones arising on the dishes were stained and counted using a low power microscope. The curves shown are representative of data obtained in several experiments.

Mutant clones having reduced activity of the purine salvage enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT; EC 2 4.2 8) were selected by their resistance to the cytotoxic purine analogue 6-thioguanine Quantitative estimation of induced mutation to purine analogue resistance is influenced by such factors as the efficiency of the chosen analogue, the concentration of that analogue, cell plating density, and time of addition of the analogue to the cells after mutagenic treatment ('expression time'). In the present experiments the optimum conditions for each of the cell types were established by varying each factor in turn until a maximum mutant frequency was found, and by reconstruction experiments[5] involving artificial mixtures of isolated mutants and wild-type cells. The results of these experiments will be published in full elsewhere. During the expression time, microcolonies are formed from the cells surviving treatment and in many previous experiments the analogue was added directly to these and not to single cells. This method does not always allow the recovery of all the induced mutants, and in the present experiments cells were maintained in exponential growth during the expression time and then replated into analogue-containing medium. The viability of the cell population was also estimated on replating, and the mutant frequencies are expressed per viable cell at this time.

Tests on several isolated human cell mutants showed that all had less than 2% of wild-type HGPRT activity (assessed by uptake of ^{14}C-hypoxanthine into whole cells[6]), and were sensitive to the glutamine analogue azaserine[7]. Only some of the hamster cell mutants, however, showed this extreme phenotype. Others had approximately 15% of wild-type HGPRT activity and could be distinguished by their ability to survive azaserine treatment. These were found not to increase in frequency with radiation dose and are excluded from the data given in Figs 2 and 3.

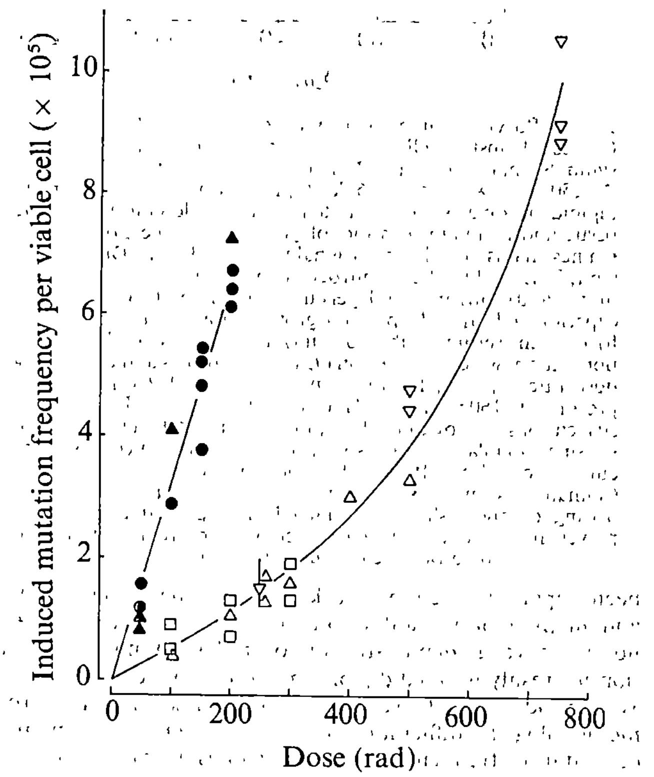

Fig. 2 Induction of HGPRT-deficient mutants of human diploid and V79 Chinese hamster cells by radiation. Symbols, growth conditions and irradiation procedures are as in Fig. 1. After irradiation, expression time growth was in normal medium for 2–3 d (hamster cells) or 7–11 d (human cells). Cells were then transferred to 9-cm plastic Petri dishes or bulk cell culture vessels (Sterilin) containing normal medium and 6-thioguanine (Sigma) at 0.7 µg ml^{-1} (hamster cells) or 2 µg ml^{-1} (human cells). Cell densities in selective medium were 2×10^3 cells cm^{-2} (hamster cells) or 10^3 cells cm^{-2} (human cells). Cells were incubated in selective medium for 12 d (hamster cells) or 21 d (human cells). Human thioguanine-resistant clones were stained and counted using a low power microscope, and hamster thioguanine-resistant clones were subjected to a second selection in azaserine medium (azaserine 2×10^{-5} M, hypoxanthine 5×10^{-5} M, thymidine 5×10^{-6} M in normal growth medium) HGPRT-negative hamster mutant clones were found to degenerate and detach from the plastic surface within 7 d of adding this medium, and were counted using dark ground illumination with a low power microscope. For a given dose, each data point shown is from a separate experiment. The symbol and vertical bar at 250 rad indicate the mean and range of 9 separate experimental determinations for hamster cells.

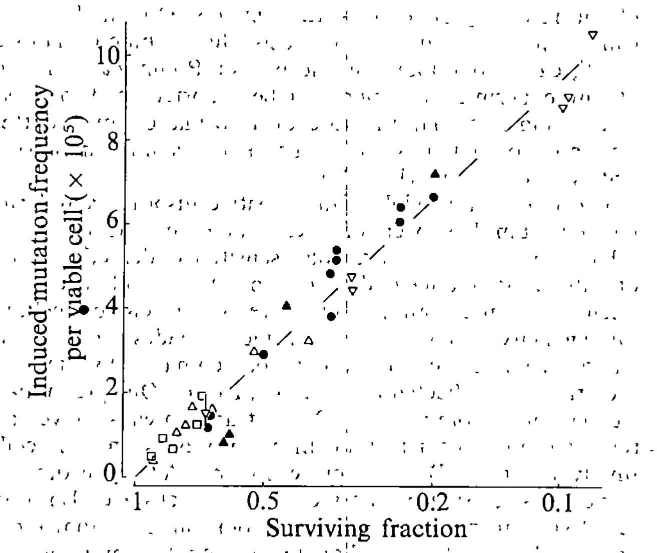

Fig. 3 The relationship between surviving fraction and induced mutation frequency in irradiated human diploid and V79 Chinese hamster cells. Symbols are as given in Fig. 1. The data points of Fig. 2 have been plotted against the log of the surviving fractions obtained in the same experiments.

Figure 1 shows the survival curves of the two cell types after ionising radiation treatment; they are clearly different, with the human cells showing an unshouldered exponential curve ($D_0 \sim 120$ rad; $n = 1$) and the hamster cells showing a shouldered response ($D_0 \sim 220$–260 rad, $n \sim 3$). These curves are similar to those previously published for radiation of low linear energy transfer[1,2,8]. The shapes of the curves of induced mutation frequency against dose are also different for the two cell types (Fig. 2), the human cells exhibiting a linear induction (4×10^{-7} mutants rad^{-1}) and the hamster cells a cumulative response (5×10^{-8} to 3×10^{-7} mutants rad^{-1}). The absolute number of mutant colonies recovered in these experiments was increased for both cell types by up to a factor of six over the dose range studied, and the pre-existing mutants were found to have no selective advantage in the conditions used.

A plot of induced mutation frequency against the logarithm of the surviving fraction for human cells must show a linear relationship, since these quantities are both linearly related to dose. A corresponding relationship was not anticipated for hamster cells, but Fig. 3 shows that it was found and, moreover, that the linear relationships shown by the two cell types were indistinguishable.

The relationships of Fig. 3 indicate that the probabilities of conversion of radiation-induced lesions to inactivation or to mutation remain in a fixed ratio, in spite of differences in the radiation response of the cell species The full implications of this finding, however, remain obscure because of our present ignorance of the molecular processes occurring in these cells after irradiation. It is possible that the survival–mutation relationship will be altered in radiosensitive mammalian cell variants, such as the cultures derived from human patients with Ataxia teleangiectasia[10], and a study of the properties of these variants may yield information on the mechanism of conversion of radiation-induced lesions.

The observed relationship between survival and the frequency of mutation to thioguanine resistance may be used to predict the frequency against dose curve for similar mutations in other cell lines from a knowledge of their survival curves alone. An extension of this approach to other mutations may be useful in predicting the genetic risk to man from exposure to ionising radiation.

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Structural alterations in fish epidermal mucus produced by water-borne lead and mercury

THE epidermal mucus of fish regulates swimming speed by controlling the hydrodynamic resistance of the skin surface[1,2]. The mucus is also presumed to serve as a defence against pathogenic organisms and is intimately associated with osmoregulation[3,4]. Because water-borne lead and mercury accumulate in the epidermal mucus of fish[5,6], it is important to ascertain whether this phenomenon produces deleterious alterations in the mucus. No detailed information is, however, available to evaluate whether such alterations occur on exposure of fish to lead and mercury or whether a capability exists to depurate these metals.

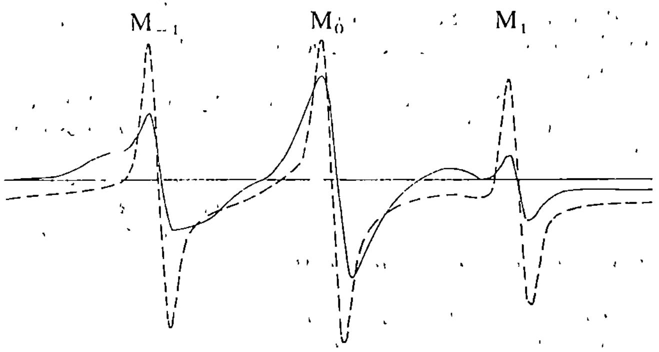

Fig. 1 Representative example of the change in electron spin resonance (ESR) spectra of fish mucus on exposure to low levels of heavy metals. ESR spectra of the control (—) and mercury-exposed (– –) mucus (1 p.p m. $HgCl_2$) labelled with N,N-dimethyl-N-dodecyl-N-tempoylammonium bromide. Spectra were obtained at 20 °C using a Varian E-3 spectrometer. The labelled samples were placed in Kimax 51 capillaries (1.6–1.8 mm diameter) The rotational correlational times were calculated using the equation:

$$\tau = \left[\left(\sqrt{\left(\frac{h_0}{h_1}\right)}+\sqrt{\left(\frac{h_0}{h_{-1}}\right)}\right)-2\right]\left[\frac{4\pi\sqrt{3}W_0}{b^2}\right]$$

where $b = 3.06 \times 10^8\ s^{-1}$; h_1, h_0 and h_{-1} are the height of peaks M_1, M_0 and M_{-1} respectively, W_0 is the width of the centre hyperfine line M_0 in Hz.

We report that small concentrations of lead and mercury in the surrounding water produce significant alterations in the properties of epidermal mucus of rainbow trout (*Salmo gairdneri*) as revealed by electron spin resonance (ESR) spectrometry. Large differences were shown to exist in the degrees of accumulation and depuration of mercury and lead, but the observed structural alterations in the mucus still remain even after a period of depuration.

The rainbow trout (350 ± 25 g) were maintained in flow-through conditions at 10 ± 0.5 °C. Experimental and control groups, each consisting of ten fish, were used. The experimental group was exposed to concentrations of $HgCl_2$ and $PbCl_2$ varying from 0.1 to 1.0 p.p.m. (µg g^{-1}). In this concentration range, the pH values (5.8 ± 0.05) of the surrounding water did not change significantly. Constant levels of metal chloride solutions were maintained by continually mixing appropriate amounts of stock solutions with fresh water using a proportionating pump (Technicon Instrument Corp., New York) at a flow rate of 2 l min^{-1}. In these initial experiments, the exposure period was chosen as the time for 20% of the fish in the experiment to die. The exposures varied from 41 to 88 h for $PbCl_2$ and 14 to 65 h for $HgCl_2$. Fish anaesthetised with dilute solutions (75 µg g^{-1}) of ethyl *m*-aminobenzoate methane sulphonate (MS-222; Crescent Research Chemicals, Inc., Scottsdale, Arizona) were suspended vertically from the mandible, and excess water was drained. The epidermal mucus (7.3 mg per g of fish) was then removed by gently scraping the skin surface with a spatula. The protein content of each sample of mucus was measured[8] and the values obtained (9.3 ± 0.9 mg protein g^{-1} mucus) were fairly constant, indicating that the mucus was obtained under reproducible conditions. Metal concentrations were determined by atomic absorption spectrophotometry[8,9]. Alterations in the properties of the mucus were evaluated by ESR using a free radical probe (N,N-dimethyl-N-dodecyl-N-tempoylammonium bromide) for polar sites. The mucus was stirred with the probe, in a ratio of 150 : 1 w/w for 12 h at 5 °C. Unassociated probe was removed by dialysis (4,000–6,000 molecular weight unit pore size) against distilled water for 12 h. By measuring the metal content of a sample before and after dialysis it was determined that this did not cause significant losses of bound lead or mercury. Rotational correlation times (τ) were calculated[10] for each ESR spectrum of mucus (Fig. 1) obtained from both control and exposed fish. The correlation time is inversely related to the tumbling frequency of the probe. A rotational correlation time of 10^{-11} s indicates rapid tumbling of the probe in a rather 'fluid' environment. A value of 10^{-8} s, however, indicates a highly immobilised probe in a relatively 'rigid' environment[10]. The rotational correlation time of 2×10^{-9} s obtained for mucus from the control fish indicates a partially immobilised probe.

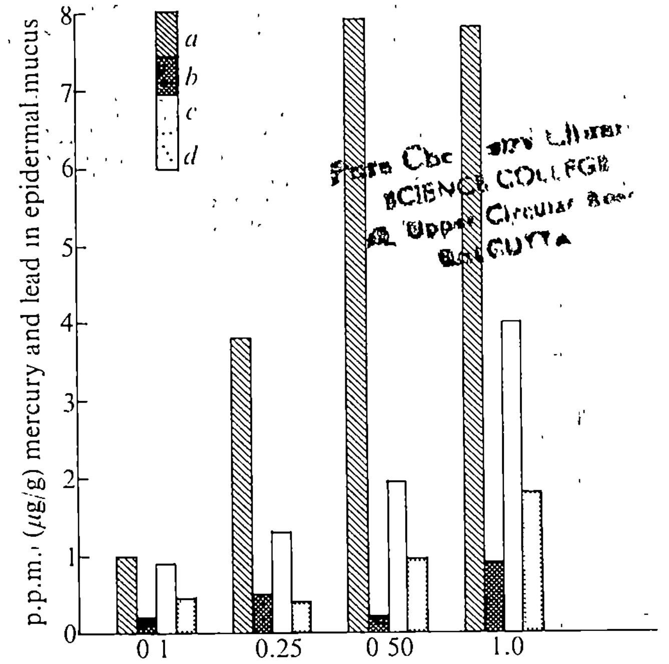

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Fig. 2 Levels of mercury and lead in the epidermal mucus of rainbow trout exposed to $HgCl_2$ and $PbCl_2$ solutions[9,10]. *a*, Accumulation of mercury in mucus after exposure to $HgCl_2$; *b*, concentration of mercury remaining in mucus after 24 h of depuration; *c*, accumulation of lead in mucus after exposure to $PbCl_2$; *d*, concentration of lead remaining in mucus after 24 h of depuration.

Exposure of fish to the aqueous $PbCl_2$ solutions showed that the mucus accumulated 0.9–4.0 p.p.m. of lead. Comparable experiments with $HgCl_2$ resulted in 1.0–7.9 p.p.m. of mercury

(Fig. 2). When the fish exposed to lead were placed in metal-free water for 24 h, the mucus depurated a maximum of 70% of the sequestered metal. In the mercury-exposed fish the maximum value obtained for the mucus depuration was 97% (Fig. 2). At each level of exposure the fish were able to depurate from the mucus a much higher percentage of mercury than lead. Accordingly, it seems that lead is more persistent in the epidermal mucus than mercury. No obvious trend exists in the degree of depuration relative to the level of exposure.

The preferential depuration of mercury seems to be related primarily to the fact that $HgCl_2$ promotes the formation of copious amounts of mucus, as shown with goldfish[6]. Our visual observations confirm this finding. The possibility that the preferential depuration results from the lead being the more tightly bound to the mucus was considered. Such an occurrence seems unlikely because our dialysis experiments with metal-exposed mucus did not result in significant losses of either mercury or lead. It seems therefore, that the decrease in the levels of lead and mercury in mucus after 24 h in metal-free waters represents a sloughing-off of the metal-complexed mucus, with subsequent replacement by metal-free mucus.

The ESR spectra of mucus obtained from the fish exposed to lead and mercury revealed that substantial alterations in rotational correlation times occurred relative to values obtained for mucus from control fish (Fig. 3). These differences were evident at each exposure level. Dramatic decreases in the τ values for concentrations between 0 and 0.1 p.p.m. of $HgCl_2$ were obtained, suggesting that the mobility of the spin label had substantially increased. Exposure of 0.1–1.0 p.p.m. of $HgCl_2$ resulted in marked increase of the τ values over the minimum value, obtained at 0.1 p.p.m. Thus, a tendency exists to reverse the trend toward the mobility of the probe at concentrations greater than 0.1 p.p.m. Nevertheless, the overall effect of the mercury was to produce a substantial increase in the fluidity of the environments around the nitroxide probe, as evidenced by much lower values obtained for the exposed mucus than for the control (Fig. 3). In essence, severe changes were induced in the structure of polar sites in the mucus by the small concentrations of inorganic mercury in the water. The ESR data for the fish exposed to lead reveals that the fluidising process was equally dramatic; however, no obvious trend in the magnitude of τ values was observed (Fig. 3). When epidermal mucus from control fish was dialysed against $HgCl_2$ and $PbCl_2$ solutions (up to 25 p.p.m.), τ values obtained were much lower than those for normal mucus[11]. Accordingly, the fluidisation observed in the metal-exposed mucus seems to be due to a direct interaction between the mucus and the metals.

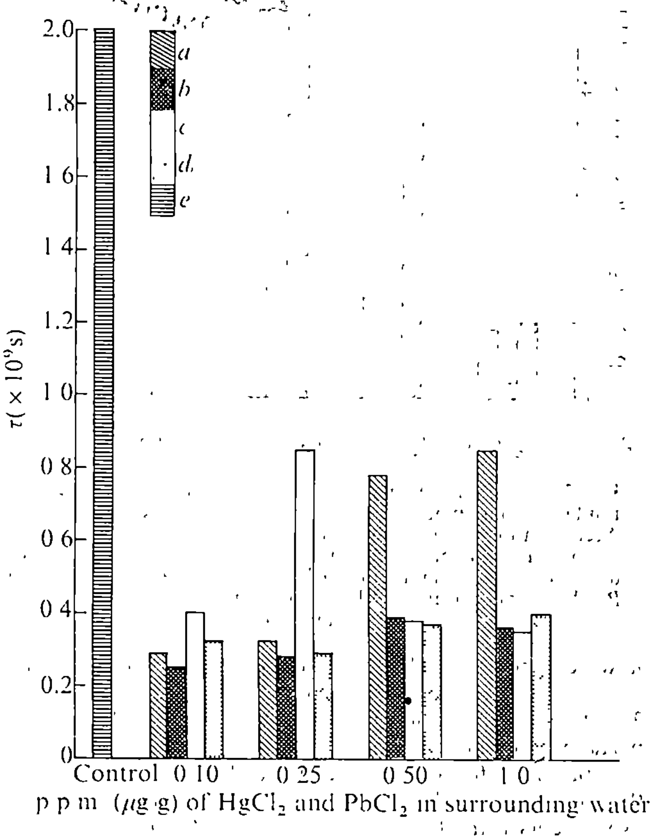

Fig. 3 Rotational correlation times (τ values) obtained from ESR spectra of epidermal mucus from control fish and metal-exposed fish. *a*, τ values for mucus of fish exposed to $HgCl_2$; *b*, τ values for mucus of mercury-exposed fish after depuration of 24 h; *c*, τ values for mucus of fish exposed to $PbCl_2$; *d*, τ values for mucus of lead-exposed fish after depuration of 24 h; *e*, τ value for mucus of control fish was 2.0×10^{-9} s.

The question arose whether the fluidisation of mucus, as shown by ESR, persists after depuration of the metals. The data given in Fig. 3 show that the τ values are still substantially lower than the control value even after 24 h of depuration. Moreover, with the exception of the 1.0 p.p.m. $PbCl_2$ exposure, the τ values were consistently lower for the depurated mucus than for the exposed mucus before depuration. Accordingly, the metal-induced damage of the mucus structure persists even after the metals are removed from the surrounding water. Further studies are required to determine whether the observed effects on the epidermal mucus are reversible over longer periods of time.

The epidermal mucus of fish contains glycoproteins as important structural units[3,12]. Thus, it seems that the observed changes may involve interactions of the metals with polar sites in these molecules. The possibility exists that the fluidisation of the mucus may alter the delicate rheological properties[1] of the epidermal mucus. Such an occurrence may result in changes in the hydrodynamic resistance and other vital processes of this medium.

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Persistence of CMV genome in lymphoid cells after congenital infection

THE persistence in a latent state of the herpes simplex virus in neuronal cells[1–3] and of the Epstein–Barr virus (EBV) in lymphoblastoid cells[4,5] has been documented. Cytomegaloviruses (CMVs) have been isolated in the past from leukocytes of congenitally infected babies[6] and found in peripheral leukocytes by *in situ* RNA–DNA cytohybridisation (C. H Huang, P. Neiman and J S. Pagano, unpublished data) EBV-positive lymphoblastoid cell lines have been established from three of 12 patients with CMV mononucleosis[7,8] but the possible persistence of CMVs in these lines was not documented. We have now found an unexpressed CMV genome in an EBV-positive lymphoblastoid cell line.

This line was established from a 9-month-old male infant (S.D.), born with a petechial rash and periventricular calcifications from a primiparous apparently healthy mother. Cytomegaloviruses were isolated from four successive urines of this baby. Three of these urines were collected from birth to age 5 months, the fourth was collected at age 19 months. Peripheral leukocytes collected successively at age 4, 9, 15 and 21 months were all established as permanent lymphoblastoid cell lines. These had B-cell characteristics, were of male karyotype and were diploid with 46 chromosomes The percentage of polyploidy of 18%, however, was slightly greater than normal. All four lines are positive for EBV soluble (S)-antigen by complement fixation and, for the EBV nuclear (EBNA) antigen by anti-complement immunofluorescence but are negative so far, even after treatment with bromodeoxyuridine for EBV viral capsid antigen[9] Cytomegalovirus antigens have not been detected using a CMV-positive EBV-negative human serum in both standard indirect and anti-complement immunofluorescence tests. Herpesvirus particules have not been seen by negative staining electron microscopy in concentrated extracts of the cells Cell extracts and supernatants for the second line did not yield CMV on human embryo fibroblast cells known to support the growth of the AD-169 CMV and the CMV from the infant's urine. The first line (EB-SD-1) contained eight EBV genome equivalents per cell as detected by DNA–DNA reassociation kinetics analysis[10], but was negative for CMV DNA by the same method[11] as well as by cRNA–DNA membrane hybridisation[11,12] (Table 1). The second line (EBCM-SD-2) was positive not only for EBNA but also for EBV early antigens and contained 8–10 CMV genome equivalents (Table 1) and 9.5 EBV genome equivalents (Fig 1) per cell. As Fig. 1 shows, however, the EBV genomes in both lines SD-1 and SD-2 only shared 65–70% homology with that of the HRIK virus.

As far as we know this is the first time that the CMV genome has been found unexpressed in a lymphoblastoid cell line. We cannot eliminate the possibility that a few non-infectious particles of CMV or CMV antigens in amounts undetectable by our immunofluorescence methods are present in these lines. To rule out the possibility that the virus in the urine could be EBV or an unusual transforming strain of CMV, we inoculated cord blood leukocytes with the virus: the cells were destroyed rapidly. The fact that EBV DNA from EB-SD-1 and EBCM-SD-2 lines shares only 65–70% of homology with EBV DNA from the HRIK line (Fig. 1) suggests either that the EBV genome in SD cells is defective or that there are different EBV strains These possibilities are supported by data showing that EBV from HRIK and from B95-8 lines can be distinguished by different biological properties[14,15] and unequal genome size[16].

Table 1 CMV and EBV genome equivalents in the cell DNA of the two first lines from infant S.D.

	Cell line	
	First (EB-SD-1)	Second (EBCM-SD-2)
CMV genome equivalents per cell		
cRNA–DNA membrane hybridisation	<1	8*, 9.5†
DNA–DNA reassocation kinetics analysis	<0.4	
EBV genome equivalents per cell		
DNA–DNA reassociation kinetics analysis	8	9.5

*^{3}H-cRNA complementary to CMV strain AD-169 DNA

†^{125}I-cRNA complementary to CMV strain AD-169 DNA.

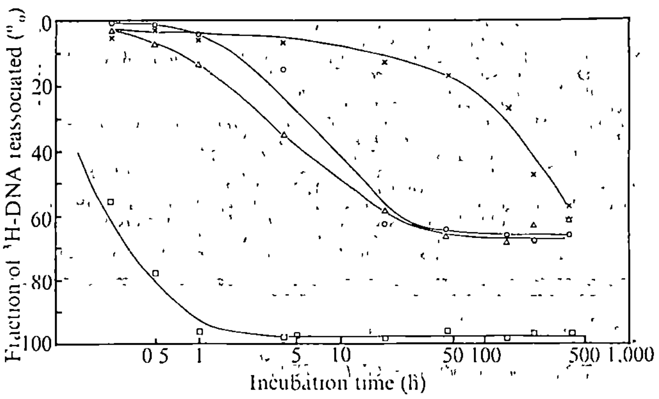

Fig. 1 DNA–DNA reassociation kinetics analysis of S.D. cell DNA and EBV ^{3}H-DNA Sonically disrupted ^{3}H-labelled EBV DNA (purified from HRIK cells), 0.02 μg (3.4 × 10^4 c.p.m.), and 2 mg of calf thymus DNA (×), EB-SD-1 cell DNA (○), EBCM-SD-2 cell DNA (△), or HRIK DNA (□) were mixed and denatured in the presence of 0 01 M Tris-HCl, pH 7.4 and 0.0025 M EDTA. The salt concentration was then adjusted to 1.2 N NaCl Hybridisaion was carried out at 66 °C, and the fraction of reassociated ^{3}H-DNA was analysed by S1 enzyme differential digestion[10].

Work is in progress to determine whether CMV played a part in the establishment of the second lymphoblastoid cell line and if it can be rescued from these cells by the co-cultivation and fusion method: Finally, labelled CMV DNA from the urine virus will be prepared and used in a reassociation kinetics assay in the presence of SD 1 and 2 cell DNA. There is no detectable (less than 5%) homology between the DNA of HRIK-EBV and AD-169 CMV[10]. Other strains of EBV and CMV, however, will have to be examined to see if they are related.

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Note added in proof: No infectious CMV could be recovered from SD2 cells following three successive cocultivation experiments with human embryo fibroblasts, but an EBNA positive lymphoblastoid cell line of female karyotype was obtained by cocultivating lethally X-irradiated SD2 male cells with cord blood lymphocytes from a female newborn. The latter experiment will be repeated using EBV positive CMV negative serum in an attempt to neutralise EBV and obtain a CMV transformed cord blood lymphocyte cell line.

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Are immunoglobulins integral membrane proteins?

IMMUNOGLOBULINS (Igs) are present on the surface of murine bone-marrow derived (B) lymphocytes[1–3]. Combination of the membrane Ig and a specific antigen, together with the arrival of a signal from thymus-derived (T) lymphocytes induces B cells to divide and differentiate into antibody secreting plasma cells[4]. To understand the mechanism of that induction, it is necessary to know how Ig is attached to the cell surface.

Singer and Nicolson[5] classified membrane proteins as integral or peripheral on the basis of their solubilities. Integral proteins, as defined by them, can only be extracted from membranes by detergents or organic solvents, and require the continued presence of these agents for solubility. Peripheral proteins can be extracted with moderate salt concentrations, slight alterations of *p*H, EDTA, and other mild reagents. Detergents are not required to maintain the solubility of peripheral proteins. Membrane Igs have been extracted with the non-ionic detergent NP-40 (refs 2 and 6) and with 8 M urea[3] but not with potassium chloride, sodium sulphate, and sodium trichloroacetate[6], and Ig remains associated with the purified plasma membrane of lymphoma cells despite extensive washing[6–8]. These findings suggest that membrane Ig may be an integral protein of the lymphocyte plasma membrane.

We sought to test whether membrane Ig was an integral protein by determining whether detergent was required for its solubility. Triton X-100 has been shown to be easily removed from protein solutions with Bio-beads SM-2 (BioRad)[9]. Since NP-40 is similar in structure to Triton X-100, we attempted to remove NP-40 from solutions with these beads. When 2.0 ml of 0.5% NP-40 in phosphate-buffered saline (PBS) were mixed with 0.6 g Bio-beads SM-2 (prepared according to Holloway's procedure[9]) and gently agitated at 4 °C for 120 min; the concentration of NP-40 in the solution declined to less than 0.001%, determined spectrophotometrically (at 277 nm). The effectiveness of detergent removal was also measured by testing the ability of solutions to haemolyse sheep erythrocytes. Using this assay, Bio-bead treatment resulted in detergent solutions of about 0.005–0.010%, probably near the critical micelle concentration of the detergent. Similar results were obtained if NP-40 (2 ml 0.5%) had been used to lyse spleen cells (1 × 10^8).

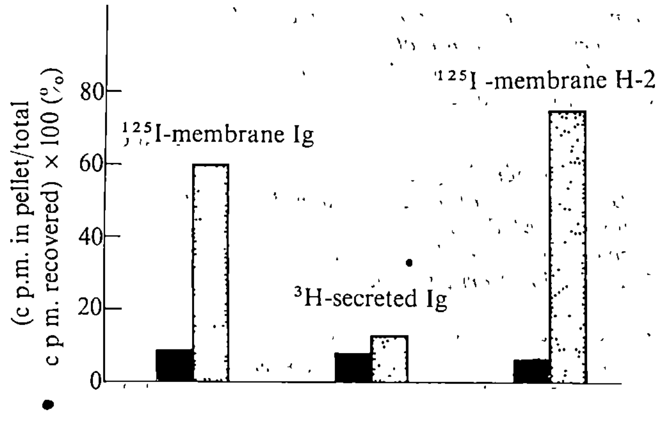

Fig. 1 Distribution of membrane Ig, secreted Ig and membrane H–2 antigen in high speed pellet for detergent present and absent. Ig was identified by sandwich immunoprecipitation with rabbit anti mouse κ, using rabbit anti-øX as control[11]. H–2 was identified in 'Ig-cleared' supernatants with ABY anti ASL-1 using C3H/BALB/c normal mouse serum as control[16]. Solid columns, NP-40 present; dotted columns, NP-40 removed.

Spleen cells from 6–12-week-old BALB/c mice were treated with Na^{125}I, lactoperoxidase and H_2O_2[2,10]; and then washed. Iodinated cells were lysed with NP-40. After removal of nuclear material and debris by centrifugation (2,000*g* for 15 min), the supernatant was divided equally into two. Bio-beads were added to one portion and both aliquots were dialysed overnight against phosphate-buffered saline (PBS) at 4 °C. The samples were freed of beads and centrifuged at 100,000*g* for 90 min. The pellet of the bead-treated sample consisted of a translucent brown layer covered by an opaque white precipitate. Only the opaque precipitate was visible in the no-bead control. The pellets were treated with 0.5% NP-40–PBS for 15 min at 37 °C, and centrifuged at 2,000*g* for 15 min to remove insoluble debris. The high speed supernatant and the NP-40 resolubilised pellet were assayed for membrane Ig by immunoprecipitation[11,12]. NP-40 was added back to the supernatant of the bead-treated sample to produce a final concentration of 0.5%, and provide similar conditions during immunoprecipitation.

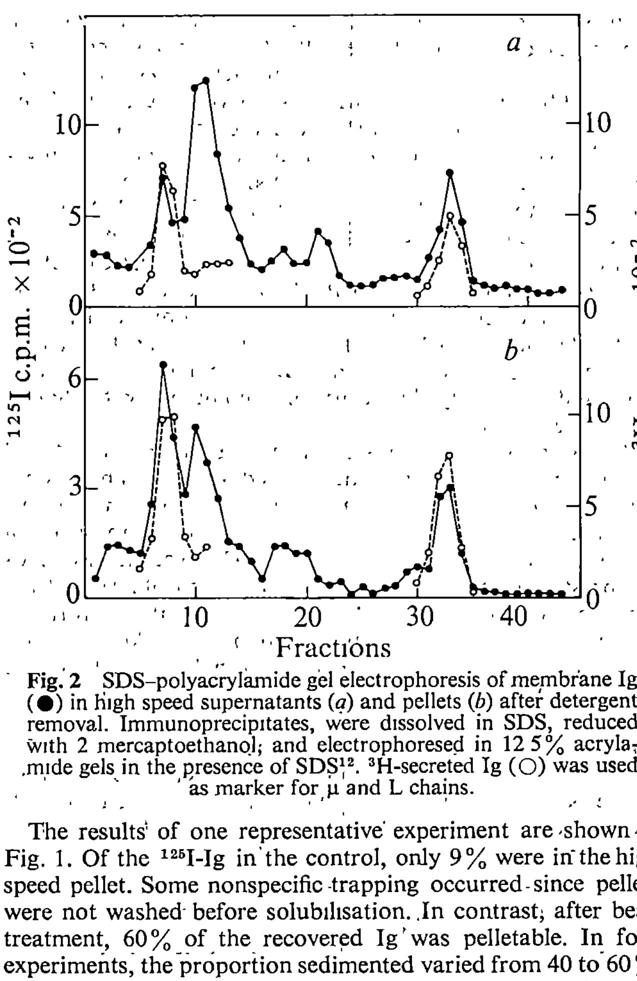

Fig. 2 SDS-polyacrylamide gel electrophoresis of membrane Ig (●) in high speed supernatants (*a*) and pellets (*b*) after detergent removal. Immunoprecipitates, were dissolved in SDS, reduced with 2 mercaptoethanol; and electrophoresed in 12.5% acrylamide gels in the presence of SDS[12]. ^{3}H-secreted Ig (○) was used as marker for μ and L chains.

The results of one representative experiment are shown in Fig. 1. Of the ^{125}I-Ig in the control, only 9% were in the high speed pellet. Some nonspecific trapping occurred since pellets were not washed before solubilisation. In contrast, after bead treatment, 60% of the recovered Ig was pelletable. In four experiments, the proportion sedimented varied from 40 to 60%. The recovery of ^{125}I-Ig in control and treated samples was always close to 100%. To control for direct effects of the beads on Ig solubility, we used Ig secreted by spleen cells. The secreted Ig had been labelled with ^{3}H by incubation of cells with ^{3}H-L-tyrosine[10,13]. The dialysed cell secretions were made to 0.5% in NP-40 and the bead treatment was the same as before. In both control and treated samples only about 10% of the ^{3}H-Ig was in the pellet fraction (Fig. 1). The remaining 90% were in the supernatant. To control for possible effects of polymerisation on the ability of pentameric IgM to aggregate, a secretion was partially reduced with 0.5 mM dithioerythritol[14]. After alkylation with 2.5 mM iodoacetamide and addition of NP-40 to 0.5%, one half of the sample was treated with beads as described above. The other half served as control.

The reduction yielded u_2L_2 and some higher polymers as determined by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis[12]. In both samples, 75% of the immunoprecipitable radioactivity was not sedimentable. Thus, the presence of detergent was required for the solubility of membrane Ig, but not for the solubility of secreted Ig either in the monomeric or polymeric forms.

That not all of the membrane Ig could be pelleted in the above experiments may indicate that sedimentation did not completely separate aggregated molecules from monomers. This view is supported by the distribution of H–2 alloantigen in the high speed pellet and supernatant. This protein, which is thought to be an integral membrane protein[15], was identified by immunoprecipitation from Ig cleared lysates[16]. Only 5% of the H–2 was pelletable in the presence of NP-40, but in its absence 75% became sedimentable (Fig. 1). That this value, like the value for membrane Ig, is not 100%, suggests that sedimentation may only separate larger sized aggregates. A preliminary analysis of a detergent-free high speed supernatant by gel filtration on Sepharose 4B showed that the membrane Ig remaining in this supernatant was present in aggregates of from 3×10^5 to 1×10^6 daltons (little or no membrane Ig radioactivity was in the 2×10^5 dalton range expected for monomeric IgM).

There are two classes of Ig on the surface of murine lymphocytes, IgM and an IgD-like molecule[11,17]. The two classes can be distinguished by the mobility of their heavy (H) chains during electrophoresis in the presence of SDS in 7.5% and higher acrylamide concentration gels. The incomplete sedimentation of membrane Ig after detergent removal could have been due to the aggregation of only one class of membrane Ig. We therefore subjected the membrane Ig immunoprecipitated from high speed supernatants and NP-40 resolubilised pellets to SDS–polyacrylamide gel electrophoresis. ^{3}H-secreted Ig was used as an internal marker for μ and light chains. As can be seen in Fig. 2, both μ and δ chains were present in the supernatant and pellet. The higher ratio of μ/δ in the pellet (and the reverse in the supernatant) compared with that of the untreated control was observed in all six experiments. The ^{3}H-μ chain markers (from secreted IgM) in Fig. 2 moved slightly faster than the membrane μ chain whether the latter was from the supernatant or the pellet. The slight difference in mobility is highly reproducible, and is discussed in detail elsewhere[12].

We believe the most likely interpretation of the above results is that IgM and IgD are integral proteins which are structurally different from their secreted highly soluble counterparts. We cannot, however, exclude an alternative but less likely possibility that membrane Ig is a peripheral protein attached by noncovalent bonds to an integral protein in the membrane. Although our results suggest that IgM is attached directly to the lipid bilayer, only a small region of the molecule, perhaps a C terminal section of the μ chains, is probably involved in this interaction, since most of the molecule is accessible to lactoperoxidase[2] and antisera[18] *in situ* on the surface of the cell.

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Circadian cycles in binding of ^{3}H-alprenolol to β-adrenergic receptor sites in rat pineal

A β-ADRENERGIC receptor coupled to adenylate cyclase regulates the activity of rat pineal N-acetyltransferase, an enzyme involved in the biosynthesis of melatonin[1]. At the onset of darkness there is an increase in the release of the neurotransmitter noradrenaline from sympathetic nerves[2,3] which produces 30- to 50-fold increases in N-acetyltransferase activity[4]. During periods of exposure to light, nerve activity and noradrenaline turnover are lower than during periods of darkness[2,3], and N-acetyltransferase activity remains at its lowest level[4].

Chronic reduction of the adrenergic input to the pineal gland, by denervation or by exposure of the animals to constant light, causes supersensitivity of N-acetyltransferase induction[5], adenylate cyclase activation[6], and cyclic AMP accumulation[5,7] to stimulation by catecholamines. Pretreatment of the animals with isoproterenol or noradrenaline prevents the development of, or reverses, this supersensitivity, causing a relative subsensitivity[8,9].

Moreover, there are daily cycles of sensitivity in the induction of N-acetyltransferase by isoproterenol[7,10] which are correlated with circadian cycles of noradrenaline turnover in the pineal gland[3]. The increase in sensitivity seen within a few hours of exposure to light is related to decreased sympathetic nerve activity, whereas the obverse is true after 12 h of exposure to darkness.

Recent work has established that ^{3}H-(−)-alprenolol (a competitive β-adrenergic antagonist) binds with high affinity and stereospecificity to pineal[11] and other tissue membranes[12]. This binding occurs at sites with characteristics indistinguishable from the β-adrenergic receptors which are coupled to adenylate cyclase. Here we report that the number of ^{3}H-(−)-alprenolol binding sites in the pineal gland varies with a circadian periodicity inversely related to the cycle of neurotransmitter release. Furthermore, these cycles in the number of specific β-adrenergic binding sites correlate with, and probably contribute to, the circadian rhythm in the sensitivity of cyclic AMP accumulation to stimulation by catecholamines.

Male Sprague–Dawley rats (150–175 g, Zivic Miller) were kept in a light/dark regime (lights on from 0600 to 1800) for at least 5 d. ^{3}H-(−)-alprenolol binding assay was performed at concentrations which saturated the specific binding sites, by a modification[11] of a method described for frog erythrocyte membranes[12]. Cyclic AMP levels were measured[13] 10 min after injection of saline or 1-isoproterenol-*d*-bitartrate (5 mg kg^{-1}, subcutaneously). The post-decapitation rise in cyclic AMP levels[14] was avoided by homogenising the pineal glands within 30 s after killing the animals.

The number of specific ^{3}H-(−)-alprenolol binding sites was measured at various times over a 24-h period, and shown to vary with diurnal periodicity (Fig. 1). The number of binding sites was lowest after 12 h in the dark. With the decrease in nerve activity that occurs at the onset of light, the number of binding sites increased. The injection of cycloheximide, an inhibitor of peptide synthesis, did not prevent the increase seen with light. Thus, the daytime reduction in β-adrenergic stimulation allows for an increase in the number of available receptor sites by a process that does not require new peptide synthesis. With the onset of darkness, the process was reversed, and the number of binding sites rapidly decreased.

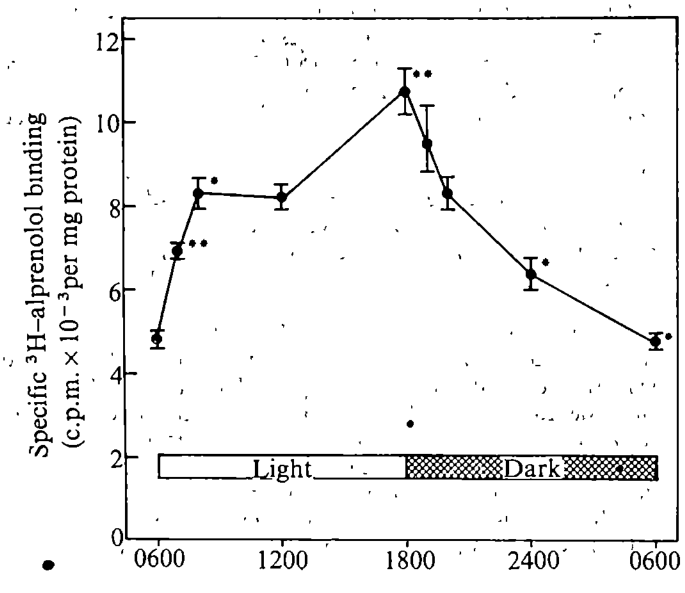
The elevation of cyclic AMP levels from baseline after an injection of isoproterenol was also examined over a 24-h period. The level of cyclic AMP in pineal glands from control animals did not vary significantly during a 24-h period (Fig. 2); the onset of light or darkness had no effect on the basal level of cyclic AMP. But the increase in cyclic AMP levels caused by the injection of isoproterenol varied markedly as a function of the previous lighting conditions. As the period of exposure to light increased, so did the accumulation of cyclic AMP after isoproterenol. With the onset of darkness, there was a decrease in the cyclic AMP response to isoproterenol measured at 2400 or 0600.

The variability of cyclic AMP accumulation after isoproterenol may be accounted for by variations either in the rate of production or destruction of cyclic AMP. Attempts to demonstrate diurnal variability in pineal phosphodiesterase activity have been unsuccessful. Small circadian variations in adenylate cyclase activation *in vitro* by catecholamines have been described previously[14] and confirmed by us. Our data show that the number of specific β-adrenergic receptor sites varies in parallel with the accumulation of cyclic AMP in response to isoproterenol. Therefore, one mechanism contributing to these diurnal cycles in the accumulation of cyclic AMP is a change in the number of β-adrenergic receptor sites, which are coupled to adenylate cyclase. These changes apparently occur as a consequence of the cycle in neurotransmitter release.

We have shown that, in the pineal gland, pretreatment with isoproterenol rapidly reduces the number of β-adrenergic receptor sites and decreases the sensitivity of adenylate cyclase to β-adrenergic stimulation[15]. These changes are in agreement with the rapid desensitisation of adenylate cyclase by catecholamines described in other systems[16,17]. In frog erythrocyte membranes such changes have also been correlated with the number of ^{3}H-(−)-alprenolol binding sites[17]. These rapid changes in the number of available β-adrenergic receptors do not require new peptide synthesis[15].

The decrease in binding of ^{3}H-(−)-alprenolol following pharmacological stimulation is not due to changes in the apparent affinities of the receptor or adenylate cyclase for agonist or antagonists[15,17]. The daily physiological variation in receptor number also is not accompanied by changes in receptor binding affinity[15]. The variation in the number of binding sites is reflected as changes in the maximum stimulation of cyclic AMP attainable, thus potentially affecting the maximum induction of N-acetyltransferase.

Fig. 1 Diurnal variation in the number of specific β-adrenergic binding sites in rat pineal gland. Each time point is the average of multiple determinations made with each of two or three homogenates containing 7–10 pooled pineals. *P <0.05; **P <0.01 compared with immediately preceding time point.



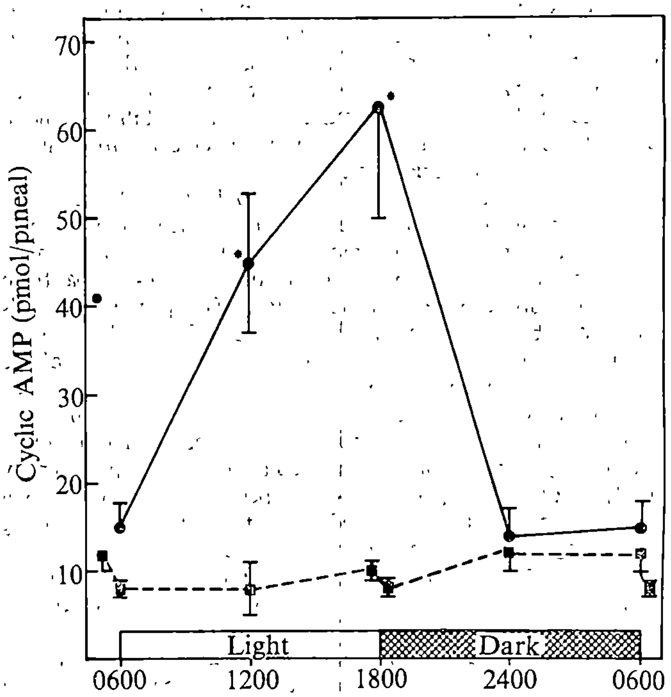


Fig. 2 Diurnal variation in the accumulation of cyclic AMP after isoproterenol administration in the rat pineal gland. At the various times indicated, rats were injected with saline (■) or l-isoproterenol-bitartrate (●) (5 mg kg^{-1}), and pineal cyclic AMP levels measured ten minutes later Results are means ± s.e.m. *P <0.05 compared with controls by Student's *t* test.

The variations in the number of β-adrenergic binding sites and in the sensitivity of the pineal gland to catecholamines are reversible and result from physiological processes. The pineal gland could thus serve as a model for studying changes in drug sensitivity as a consequence of endogenous processes[18]. Furthermore, since the changes can also be produced by pharmacological manipulations, investigations in the pineal may have bearing on the phenomena of tachyphylaxis and drug tolerance.

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Direct identification and characterisation of β-adrenergic receptors in rat brain

MODULATION of cellular function by the autonomic nervous system is accomplished, at least in part, by the stimulation of β-adrenergic receptors by catecholamines at neuroeffector junctions[1]. Activation of these receptors results in stimulation of adenylate cyclase with increased intracellular levels of cyclic AMP. The β-adrenergic receptors of a variety of non-neural tissues have been characterised indirectly by measurement of altered adenylate cyclase activity or of intracellular cyclic AMP in response to β-adrenergic agonists and antagonists. Direct characterisation of the β-adrenergic receptors in avian[2,3] and amphibian[4] erythrocytes and in canine heart[5] has been accomplished by binding assays using radioactive β-adrenergic antagonists.

Catecholamines are thought also to be neurotransmitters mediating information transfer across synapses in the central nervous system (CNS)[6]. In the case of dopaminergic systems this mediation is thought to result from the stimulation of a dopamine receptor-coupled adenylate cyclase resulting in increased levels of cyclic AMP[7]. This dopamine-sensitive adenylate cyclase system has been extensively characterised in terms of structure–activity relationships and interactions with psychotropic drugs[8]. In contrast, although β-adrenergic receptor mediated changes in cyclic AMP have been observed in brain slices[9] and electrophysiological evidence suggests that these receptors are important in mediating function in certain neurones[10–12], relatively little information is available concerning β-adrenergic receptor systems in the brain.

The β-adrenergic receptor of the CNS has not been fully characterised even by indirect methods because of the relative difficulty of measuring physiological responses and because, in contrast to most other tissues, the β-adrenergic receptor coupled adenylate cyclase in brain generally retains little or no hormone sensitivity after homogenisation[13]. Because of success in using ^{3}H-(−)-alprenolol, a β-adrenergic antagonist, to identify β-adrenergic receptors in canine myocardium and in frog erythrocytes, we used this compound to identify and to characterise the β-adrenergic receptor of the rat CNS.

Male Sprague–Dawley rats (225–300 g) were killed by decapitation. The brain was quickly removed, dissected and frozen at −70 °C until used. Brain sections were homogenised in 5–8 volumes of cold buffer (0.25 M sucrose, 5 mM Tris-HCl, *p*H 7.4 and 1 mM $MgCl_2$) using 5–6 passes with a tight fitting Dounce homogeniser. The homogenate was centrifuged at 800*g* for 20 min at 4 °C and the pellet was discarded. The supernatant was centrifuged at 4 °C for 14 min at 14,000*g* and the resulting pellet was washed three times in the sucrose buffer. The pellet was resuspended in cold buffer (75 mM Tris-HCl, 25 mM $MgCl_2$, *p*H 7.4) to a final protein concentration of 200–300 µg per 100 µl. Incubation of the crude membrane fraction, 18 nM ^{3}H-(−)-alprenolol (specific activity 17 Ci mmol^{-1}), and various agonists and antagonists were performed in a volume of 150 µl for 10 min at 37 °C as described previously[5]. The binding reaction was terminated by rapidly adding 3 ml of ice cold buffer (50 mM Tris-HCl, 17 mM $MgCl_2$, *p*H 7.4) and filtering through a Whatman GFC glass fibre filter mounted in a suction apparatus. The incubation tube was washed with additional 3 and 5-ml portions of cold buffer. The filter was subsequently dried and the trapped particles with their bound radioactivity were counted in a liquid scintillation counter using a Triton X-100 on toluene based scintillation fluid. The filter blank was less than 1% of added counts. "Specific" binding (counts displaced by 1×10^{-6} or 1×10^{-5} M (−)-propranolol) averaged approximately 70% of total bound counts. "^{3}H-(−)-alprenolol binding" refers to specific binding.

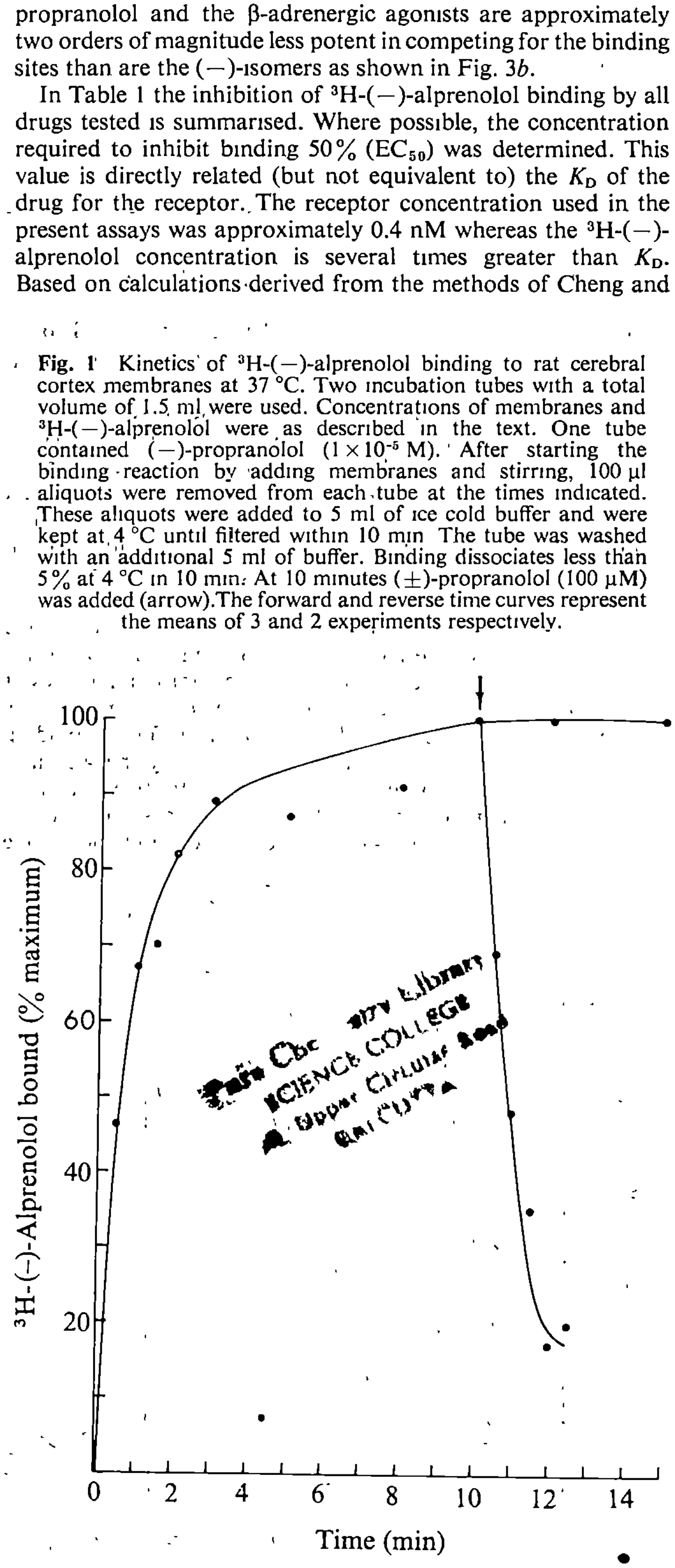
Figure 1 shows the kinetics of ^{3}H-(−)-alprenolol binding to the cerebral cortex membrane fraction. Binding is approximately 50% complete at the earliest time measured (30 s) and is virtually at equilibrium by 3 min. The reverse rate is also very rapid with a $t_{\frac{1}{2}}$ of approximately 30–60 s at 37 °C.

Figure 2 shows that there are a finite number of specific binding sites in the rat cerebral cortex membrane fraction, as shown by the saturability of ^{3}H-(−)-alprenolol binding. Half maximal saturation occurred at 6.7 nM which provides an estimate of the dissociation constant (K_D) of (−)-alprenolol for the binding sites.

The specificity of ^{3}H-(−)-alprenolol binding is demonstrated in Fig. 3*a*. Thus (−)-propranolol is highly potent in competing for the binding sites. The order of potency of the β-adrenergic agonists in competing for the binding sites is (−)-isoproterenol > (−)-adrenaline = (−)-noradrenaline. The (+)-isomers of propranolol and the β-adrenergic agonists are approximately two orders of magnitude less potent in competing for the binding sites than are the (−)-isomers as shown in Fig. 3*b*.

In Table 1 the inhibition of ^{3}H-(−)-alprenolol binding by all drugs tested is summarised. Where possible, the concentration required to inhibit binding 50% (EC_{50}) was determined. This value is directly related (but not equivalent to) the K_D of the drug for the receptor. The receptor concentration used in the present assays was approximately 0.4 nM whereas the ^{3}H-(−)-alprenolol concentration is several times greater than K_D. Based on calculations derived from the methods of Cheng and

Fig. 1 Kinetics of ^{3}H-(−)-alprenolol binding to rat cerebral cortex membranes at 37 °C. Two incubation tubes with a total volume of 1.5 ml were used. Concentrations of membranes and ^{3}H-(−)-alprenolol were as described in the text. One tube contained (−)-propranolol (1×10^{-5} M). After starting the binding reaction by adding membranes and stirring, 100 µl aliquots were removed from each tube at the times indicated. These aliquots were added to 5 ml of ice cold buffer and were kept at 4 °C until filtered within 10 min. The tube was washed with an additional 5 ml of buffer. Binding dissociates less than 5% at 4 °C in 10 min. At 10 minutes (±)-propranolol (100 µM) was added (arrow). The forward and reverse time curves represent the means of 3 and 2 experiments respectively.



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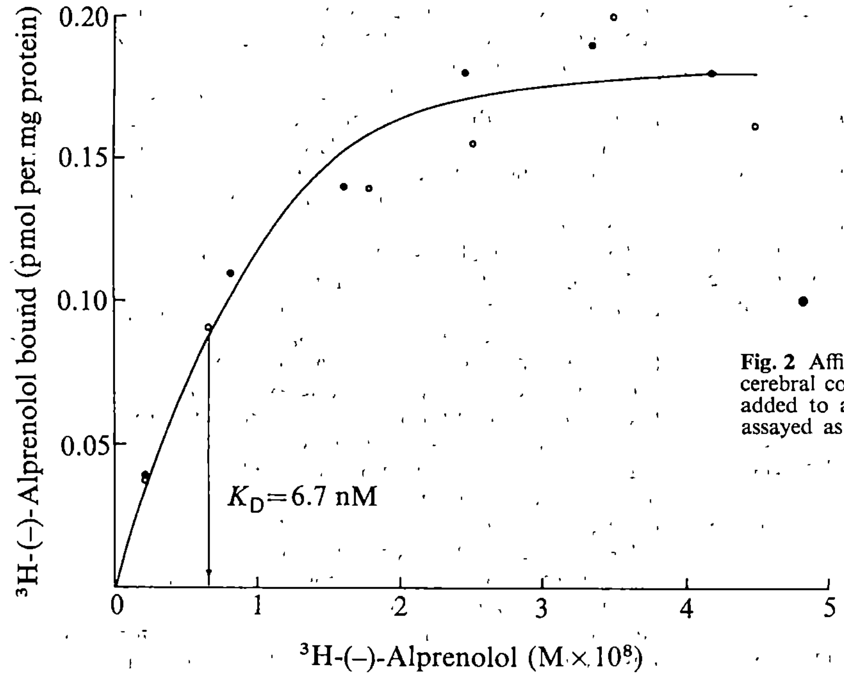

Fig. 2 Affinity and saturability of ^{3}H-(−)-alprenolol sites in rat cerebral cortex. Increasing amounts of ^{3}H-(−)-alprenolol were added to a fixed amount of membrane protein. Binding was assayed as described in the text. Two experiments are shown.

Prusoff[14] and Rodbard and Lewald[15] the EC_{50} values in Table 1 are approximately four- to tenfold higher than the actual K_D values. It should be stressed that as the receptor and ^{3}H-(−)-alprenolol concentrations in the assays decrease, the EC_{50} values for various ligands will also decrease and ultimately approach their true K_D values. Thus, the observed EC_{50} in the binding assay is only a relative measure of the affinity of an agent for the receptor which is influenced by a variety of technical factors. The K_D for any agent, which may be calculated from the EC_{50} (refs 14 and 15) is, however, a constant presumably independent of assay conditions.

As may be expected from their similarity to β-adrenergic agents (ethylamine side chain) dopamine and 5-hydroxytryptamine (5-HT) competed for ^{3}H-(−)-alprenolol binding sites at high concentrations. Catecholamine precursors or metabolites and octopamine compete weakly or not at all for the binding sites. Other putative neurotransmitters such as glycine, 4 aminobutyric acid, and histamine are only weakly effective or ineffective in competing for binding. Pyrocatechol does not compete for the binding sites. The α-adrenergic antagonist phentolamine displaces 50% of specifically bound alprenolol at 1×10^{-4} M.

The distribution of ^{3}H-(−)-alprenolol binding sites is compared with the concentration of noradrenaline in different regions of the rat CNS in Table 2. The areas highest in binding sites, the cerebral cortex, limbic forebrain and corpus striatum, had concentrations of binding sites comparable with the number of binding sites in canine myocardial membranes, a tissue rich in β-adrenergic receptors[5]. The hypothalamus and adjacent diencephalon had high noradrenaline content but relatively fewer ^{3}H-(−)-alprenolol binding sites. The cerebellum and hippocampus, areas where β-adrenergic receptors have been identified electrophysiologically[16,17], have approximately the same proportion of binding sites and noradrenaline. In spite of substantial amounts of noradrenaline in the spinal cord and medulla, little specific ^{3}H-(−)-alprenolol binding was observed in these regions.

The ^{3}H-(−)-alprenolol binding sites in rat brain have characteristics similar to those described for β-adrenergic receptors in other tissues such as the heart[5], frog erythrocytes[4] and human lymphocytes[18]. Binding is rapid and reversible,

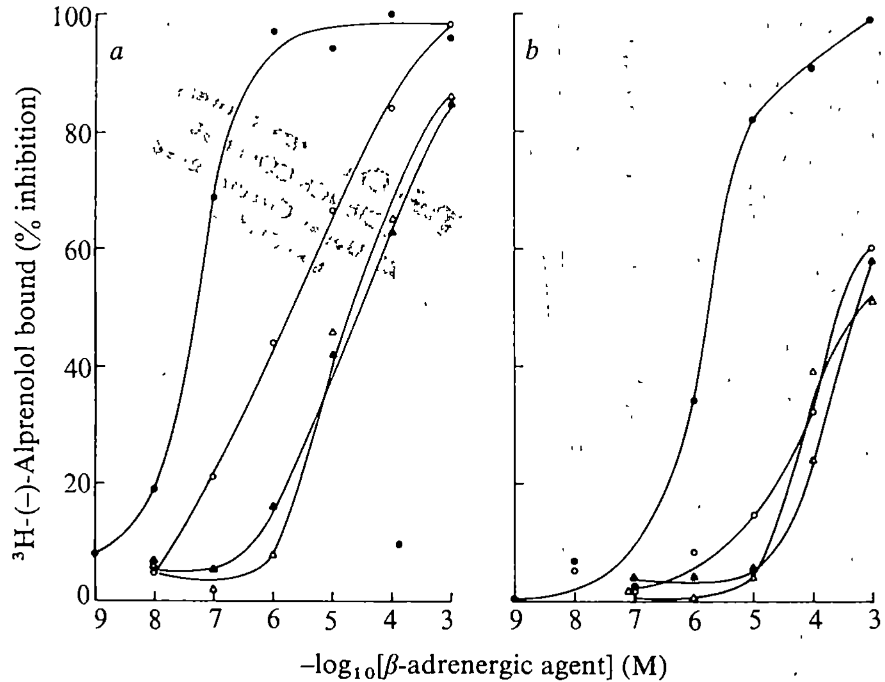

Fig. 3 Specificity of ^{3}H-(−)-alprenolol binding in rat cerebral cortex membranes. Dose–response curves for the inhibition of ^{3}H-(−)-alprenolol binding by the (−)-stereoisomers of propranolol (●), isoproterenol (○), noradrenaline (▲), and adrenaline (△) are shown in *a*. The inhibition of ^{3}H-(−)-alprenolol binding by the (+)-stereoisomers of these agents is shown in *b*. The symbols are identical to those used in *a*.

stereospecific, saturable and exhibits high affinity for β-adrenergic antagonists. These features suggest that the binding sites in the present study represent physiological β-adrenergic receptors. The catecholamine potency series defines these receptors as being of the β_1 subtype[19].

The ability of the α-adrenergic antagonist phentolamine to displace ^{3}H-(−)-alprenolol at high concentrations is compatible with the "nonspecific" inhibitory effects of high concentrations of phentolamine on β-adrenergic physiological responses[20] and on β-adrenergic receptor coupled adenylate cyclase in other tissues[21]. This nonspecific effect on the β-adrenergic receptor of rat brain suggests that the designation, based on phentolamine inhibition, of catecholamine effects in the rat brain as being α-adrenergic receptor-mediated be approached with caution.

The displacement of ^{3}H-(−)-alprenolol by 5-HT at 10^{-4} M is not surprising in view of the fact that 5-HT at high concentrations has been shown to inhibit catecholamine stimulation of β-adrenergic receptor coupled adenylate cyclase in avian erythrocytes[22] and rat pineal[23] and to inhibit noradrenaline induced cyclic AMP accumulation in various areas of the rat brain[24]. The present data do not permit a conclusion as to whether 5-HT interacts with the β-adrenergic receptor of rat cerebral cortex as an antagonist or perhaps as a partial agonist.

There was no apparent correlation between levels of noradrenaline and number of β-adrenergic receptors in various brain regions. Similar lack of correlation between levels of transmitter and number of receptors for that transmitter has been observed in other instances as has been reviewed[25]. Possible explanations include variations in different brain regions of: (1) density of noradrenergic innervation of neurones; (2) noradrenaline content of nerve terminals; and (3) relative surface area of the postsynaptic membrane associated with noradrenergic nerve terminals. In addition, noradrenaline nerve terminals could be associated with α-adrenergic postsynaptic receptors.

Noradrenaline has an inhibitory effect on the discharge of Purkinje cells in the rat cerebellum[10,11]. This inhibitory effect is mediated through a β-adrenergic receptor–adenylate cyclase system since the effects are mimicked by the iontophoretic application of cyclic AMP and since β-adrenergic antagonists block the effects of noradrenaline but not those of cyclic AMP. Inhibition of discharge rates by noradrenaline and cyclic AMP has also been observed in pyramidal tract neurones of the rat cerebral cortex[12].

Table 1 Effects of various agents on ^{3}H-(−)-alprenolol binding to rat cerebral cortex membranes

Agent	Half-maximum inhibition of ^{3}H-(−)-alprenolol binding (μM)
(−)-Propranolol	0.056
(+)-Propranolol	1.4
(−)-Isoproterenol	2.0
(−)-Noradrenaline	16.0
(−)-Adrenaline	16.0
Phentolamine	100.0
5-HT	100.0
(+)-Isoproterenol	440.0
(+)-Adrenaline	708.0
(+)-Noradrenaline	900.0
Dopamine	*
(±)-Octopamine	*
(±)-Normetanephrine	*
(±)-3,4-dihydroxymandelic acid	NI
(±)-Dihydroxyphenylalanine (dopa)	NI
Tyramine	NI
Pyrocatechol	NI
Glycine	NI
(−)-Aspartic acid	NI
(−)-Glutamic acid	NI
Histamine	NI
4-Aminobutyric acid	NI

^{3}H-(−)-alprenolol binding was assayed as described in the text. Maximum inhibition of binding is defined as the binding inhibited by 10^{-6} M (−)-propranolol. *Less than 50% inhibition of binding at 1×10^{-4} M; NI, no inhibition of binding at this concentration.

Table 2 Distribution of ^{3}H-(−)-alprenolol binding sites and noradrenaline in the rat CNS

Brain area	^{3}H-(−)-Alprenolol binding sites (pmol/mg protein)	Noradrenaline (ng/g frozen tissue)
Limbic forebrain	0.28±0.06 (4)	772±44 (3)
Hippocampus	0.26±0.02 (4)	417±28 (4)
Parietotemporal cortex	0.23±0.02 (4)	350±7 (3)
Corpus striatum	0.22±0.02 (4)	162±35 (4)
Frontal cortex	0.20±0.03 (4)	428±44 (4)
Diencephalon	0.20±0.03 (4)	674±66 (4)
Hypothalamus	0.15±0.01 (4)	1,652±145 (4)
Pons	0.11±0.01 (4)	649±42 (4)
Cerebellum	0.11±0.02 (4)	207±18 (4)
Medulla	0.06±0.02 (4)	342±20 (4)
Spinal cord	0.06±0.02 (6)	423±41 (8)

Fresh rat brains were dissected on an iced Petri dish with fine forceps. The corpus callosum was split and the two cerebral cortices were splayed by opening the lateral ventricles. Using the anterior commissure as a landmark, the limbic forebrain (septal nuclei, nucleus accumbens and olfactory tubercles) was dissected from the diencephalon. The corpus striatum was next dissected and then the fornix was divided. The hippocampus, parietotemporal cortex, and frontal cortex were then separated. The cerebellum was removed from the remaining brainstem and the medulla was separated from the pons at the pontomedullary junction. The brainstem was inverted and the hypothalamus was removed by dissection of the grey matter surrounding the median eminence from the surrounding white matter. The pons was separated from the rest of the brainstem which was designated as the diencephalon. Each brain region was frozen on dry ice. For the noradrenaline assays, regions from three animals were pooled for each determination. ^{3}H-(−)-alprenolol binding sites were assayed by adding saturating levels of ^{3}H-(−)-alprenolol with or without 10^{-6} M (−)-propranolol to membranes and assaying as described in the text. Noradrenaline was assayed by a fluorometric method as described previously[28]. Values represent mean±s.e.m. followed by the number of experiments in parentheses. Each point was assayed in duplicate.

It is tempting to postulate that the binding studied in the present investigation represents postsynaptic β-adrenergic receptors involved in information transmission between neurones. Such a speculation requires further substantiating evidence. This is especially true in view of the fact that a β-adrenergic receptor coupled adenylate cyclase has been reported in various glial cell cultures[26,27]. Thus, the anatomical localisation of the ^{3}H-(−)-alprenolol binding sites in the brain requires further investigation.

Direct binding with ^{3}H-(−)-alprenolol provides a new technique for studying the role of the β-adrenergic receptor in the neurophysiology and pathophysiology of the CNS. In view of the technical difficulty in maintaining catecholamine responsiveness of adenylate cyclase in brain homogenates[13], these binding techniques may ultimately facilitate the localisation of sites of hormonal responsiveness in the CNS. The direct study of the regulation of the brain β-adrenergic receptor as a physiological entity is also now possible.

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Prolonged activation of tyrosine hydroxylase in noradrenergic neurones of rat brain by cholinergic stimulation

IN sympathetic ganglia and adrenal medulla, the augmented release of acetylcholine from preganglionic sympathetic neurones can lead to prolonged changes in the activity of tyrosine hydroxylase (TH), the enzyme catalysing the rate-limiting step in the biosynthesis of catecholamines[1,2]. The trans-synaptically mediated increase in TH activity is due to excitation of cholinergic receptors[3–5], occurs after a latency of many hours to days, remains elevated up to weeks and is attributable entirely to increased accumulation of TH enzyme molecules; it therefore represents induction of the enzyme.

In brain, a three- to fourfold increase in the activity and amount of TH, comparable in many respects to the trans-synaptic induction of the enzyme observed in ganglia and adrenals, can be initiated in the cell bodies of noradrenergic neurones of the locus coeruleus by reserpine[6,7] Since the locus coeruleus contains large amounts of enzymes subserving the synthesis and degradation of acetylcholine[8,9], we wondered whether stimulation of cholinergic receptors would induce TH in this area of the brain as it does in the periphery.

We therefore set out to determine if the centrally active cholinergic agonist, oxotremorine, which stimulates muscarinic receptors[10] can induce TH in the locus coeruleus. We report that the administration of oxotremorine to rats results in a prolonged elevation of TH activity in the locus coeruleus, similar in time course to that elicited by reserpine The biochemical basis for the increase in enzyme activity is, however, entirely different from that produced by reserpine, being entirely attributable to prolonged increase of the catalytic activity of the enzyme molecules (activation) This mode of regulation of TH, which we have termed delayed activation, seems not to have been recognised before.

Oxotremorine (Aldrich Chemical Company, Inc) was administered to male Sprague–Dawley rats weighing 225–275 g, in doses from 0 25 to 2 5 mg kg⁻¹ subcutaneously Controls received saline. The animals were killed by cervical dislocation on various days thereafter, and the region of the locus coeruleus was dissected[11] and homogenised in 10 volumes of 0 005 M phosphate buffer containing 0 2% Triton X-100. Tyrosine hydroxylase was assayed by a modification of the method of Coyle[12], with omission of quinonoid dihydropterin reductase and using mercaptoethanol for stabilisation of the cofactor $DMPH_4$ (6,7-dimenthyl-5,6,7,8-tetrahydropteridine). Saturating concentrations of tyrosine and $DMPH_4$ were used. Enzyme activity was calculated as pmol dopa formed per h per pair of loci coerulei for each animal.

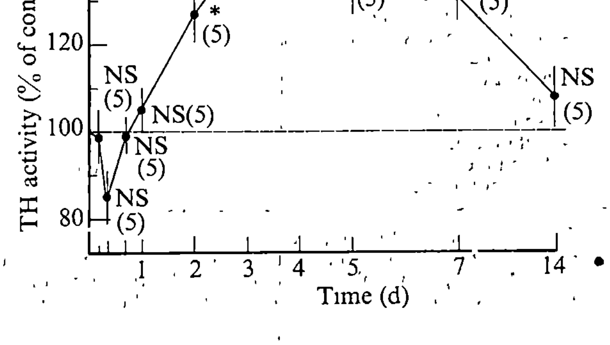
A single injection of oxotremorine (1.5 mg kg⁻¹) resulted in a prolonged increase in the activity of TH in the locus coeruleus (Fig. 1). The increased activity appeared after a latency of 24 h, rose to a maximum of 40% of control at 72 h after the injection and returned to control approximately 14 d later. The increase was dose dependent with a threshold response of 0.5 mg kg⁻¹, and seemed specifically related to activation of muscarinic cholinergic receptors since it was blocked by atropine (40 mg kg⁻¹, administered intraperitoneally 30 min before oxotremorine).

We next sought to determine by immunotitration with a specific antibody to TH[6,13], if the increase in enzyme activity in the locus coeruleus produced by oxotremorine was due to increased accumulation of enzyme molecules or to activation of the existing enzyme. Immunochemical titration was performed by our adaption[13] of the method of Feigelson and Greengard[14], using specific antibodies to bovine adrenal TH prepared as described elsewhere[6,13]. Two immunotitration procedures were used. In the first, increasing amounts of tissue from control and treated animals were added to a fixed amount of antibody, the antibody–enzyme complex was precipitated out, and enzyme activity remaining in the supernatant was determined. The concentration of tissue (enzyme) that precipitates out all the antibody is termed the equivalence point and is graphically identified as the point on the abscissa at which enzyme activity first appears in the supernatant.

In the second immunotitration procedure, the tissue extract of treated animals was diluted so that the enzyme activity in an aliquot was identical to that in an equal volume of homogenate from controls.

The results of the first experiment are illustrated in Fig. 2*a* and demonstrate that the immunotitration curves of the locus coeruleus of oxotremorine-treated animals was steeper than that of saline-injected controls, but shared an identical equivalence point. This observation indicates that the number of immunoreactive molecules of TH in the locus coeruleus in treated animals is the same as for controls; however, the catalytic activity of each enzyme

Fig. 1 Time course of the changes in tyrosine hydroxylase (TH) activity in the locus coeruleus of the rat after injection of oxotremorine (1 5 mg kg⁻¹ subcutaneously). Values (mean ± s.e.m.) are expressed as percentages of controls. Control values were (mean ± s.e m.; $n = 13$) 1,627 ± 49 pmol of dopa per pair of locus coeruleus. Figures in brackets represent number of observations. * $P < 0\,001$ compared with controls; NS, not significant.



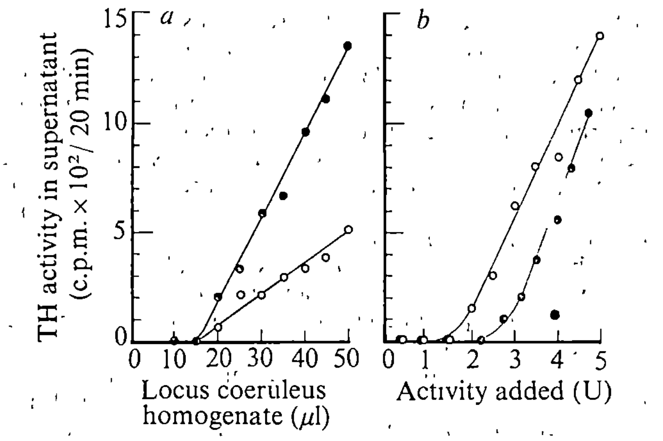


Fig. 2 Immunotitration of tyrosine hydroxylase (TH) in the locus coeruleus with an antibody to purified TH from bovine adrenal gland Homogenates of brain tissue from the region of the locus coeruleus were prepared, from rats treated with oxotremorine (1.5 mg kg^{-1} subcutaneously once daily for 3 d; the rats were killed at d 6) or saline injected controls. ●, Oxotremorine; ○, control. *a*, A fixed amount of antibody (15 μl of antiserum) was added to test tubes containing increasing concentrations of supernatant (6,000*g*; 10 min) of tissue homogenate (0–50 μl in steps of 5 μl) diluted with homogenising buffer to give a constant volume of 50 μl in each tube. After incubation for 1 h at room temperature and subsequent centrifugation, TH activity was measured in the supernatant. Note that the same equivalence point was obtained for the oxotremorine- and saline-treated rats. *b*, Supernatants of locus coeruleus tissue homogenates from oxotremorine-treated rats were diluted with homogenising buffer and adjusted to give the same TH activity as in the supernatant of locus coeruleus tissue homogenate from control rats Antiserum (15 μl) was added to test tubes containing increasing amounts of tissue extracts from the oxotremorine- and saline-treated groups respectively in a total volume of 50 μl. After incubation of the antibody–antigen mixture for 1 h at room temperature, the tubes were centrifuged and the TH was measured as the amount of ^{14}C-dopa (c.p.m.) formed per 20 min per 50 μl of supernatant Note that the oxotremorine treatment shifted the immunotitration curve to the right. The results indicate that oxotremorine caused an increase in activity of enzyme molecules rather than causing an accumulation of more enzyme protein.

molecule is increased. The second experiment (Fig. 2*b*) was confirmatory. Comparison of the immunotitration curves of homogenates of locus coeruleus of treated and control animals when adjusted for equal activity per unit volume, demonstrated that enzyme activity is increased without apparent increase in the amount of enzyme protein.

This study demonstrates that administration of the cholinergic agonist, oxotremorine, can increase the activity of tyrosine hydroxylase in central noradrenergic neurones of the locus coeruleus The response occurs as a consequence of stimulation of central muscarinic receptors, either on locus coeruleus neurones or on interneurones, since it is blocked by atropine. The increase in TH activity elicited by oxotremorine is comparable in many respects to that produced by reserpine: it occurs after a latency of 24 h, reaches a peak (although smaller in magnitude) by 72 h and remains elevated for two weeks. But the immunochemical experiments show that, in contrast to reserpine, oxotremorine increases TH activity by activation rather than accumulation of enzyme molecules.

The delayed activation of TH by oxotremorine clearly differs from other modes of activation of the enzyme, such as those observed in terminals of locus coeruleus neurones by electrical stimulation[15], or in terminals of central dopaminergic neurones, after lesions[16] or administration of pharmacological agents including reserpine or neuroleptics[17]. These modes of activation have their onset within minutes of the stimulus, disappear within hours, and are associated with changes in the kinetics of the enzyme, either with respect to substrate[15,17] or cofactor[17]. In contrast, the cholinergically activated enzyme not only has a prolonged time course, but fails to demonstrate changes in the K_m for substrate or cofactor; an increased V_{max} being the only alteration in kinetics (T.H.J., T.L. and D.J.R., unpublished observations).

This cholinergically-mediated delayed activation of tyrosine hydroxylase therefore seems to represent a previously unrecognised mode of regulation of the enzyme. Its detection has been made possible only by the application of immunochemical techniques. The biochemical mechanisms underlying the changes in enzyme activity are unknown. The time course of the change is consistent with some process requiring the biosynthesis of macromolecules. One possibility would be that in response to the drug a different and catalytically more active form of the enzyme is synthesised; another mechanism, consistent with the kinetic data would be that a non-competitive inhibitor of the enzyme is removed. The fact that comparable activation of TH can be elicited by physostigmine (T.L., T.H.J. and D.J.R., unpublished), an agent which facilitates the action of naturally released acetylcholine by blocking its degradation, however, suggests that delayed activation of TH may be a naturally occurring mode of regulation of the enzyme The functional significance of this mode of regulation and its possible role in mediating the action of centrally active agents acting on noradrenergic transmission remain to be established.

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Oestradiol receptor of neonatal mouse brain

ADMINISTRATION of oestrogen or androgen to neonatal rats or mice can cause sterilisation of females[1]. Adult sterilised females generally exhibit masculinised behaviour and do not have normal cyclic gonadotropin patterns. Are prospective receptors for oestrogens and androgens present in neonatal rat or mouse brain tissues that are responsive to sex steroids? Direct demonstration of oestradiol receptor in brains of neonatal rats has been made difficult by the "neonatal binding protein" (NBP)[2,3] that binds oestradiol with much higher capacity but lower affinity than does presumed receptor. If oestradiol receptor is present and functional, does NBP act in neonatal brain to prevent sterilisation by maternal oestrogens?[3]

Making use of the affinity for DNA of presumed oestradiol receptor from hypothalamus–preoptic area of prepubertal mice[4], I have now separated this binding complex

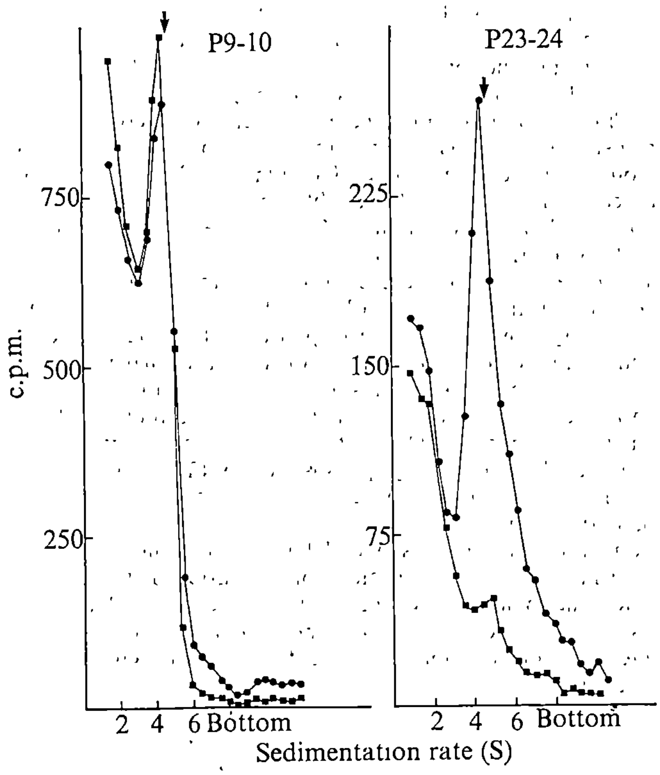


Fig. 1 Sucrose gradient sedimentation of bound ^{3}H-oestradiol incubated alone and with DES in extracts of hypothalamus–preoptic area from second and fourth week mice. As before[1,4], seven fourth week (postnatal days = P23–24) females (C57BL/6J × C3H/HeJ) F$_1$ hybrid mice were anaesthetised with ether, killed by saline perfusion and dissected to yield a block (wet weight, 0.30 g) of hypothalamus–preoptic area. The tissue was homogenised as described[1,4] in buffer containing 0.01 M Tris-HCl, *p*H 8.1 (21 °C), 1 mM Na$_3$EDTA (ethylenediamine tetraacetate), 1 mM mercaptoethanol, 0.15 M NaCl and 10% (v/v) glycerol and then centrifuged to obtain a clear supernatant. Seven second week (postnatal days = P9–10) female F$_1$ hybrid mice were etherised, decapitated and dissected (tissue wet weight, 0.45 g). Young animals in this and the following experiments were treated in this manner to optimise presumed receptor yield by minimising preparation time. Aliquots of each extract were incubated with 4.5 nM ^{3}H-oestradiol (110 Ci mmol^{-1}, New England Nuclear) alone (●) or with 300 nM (×67) DES (■). Labelled extracts were freed of unbound oestradiol on Sephadex G-25 columns and then sedimented in 5–20% (w/v) sucrose gradients. The arrow indicates the internal fluorescent marker, dansyl-bovine serum albumin (sedimentation rate 4.7S).

from extracts of mice 2–12 d old. This fractionation directly demonstrates the existence of adult-like oestradiol receptor in neonatal mouse brain and permits examination of its binding characteristics in the presence of NBP. This technique enables me to test the suggested function of NBP and will facilitate quantitative studies of the separated receptor itself.

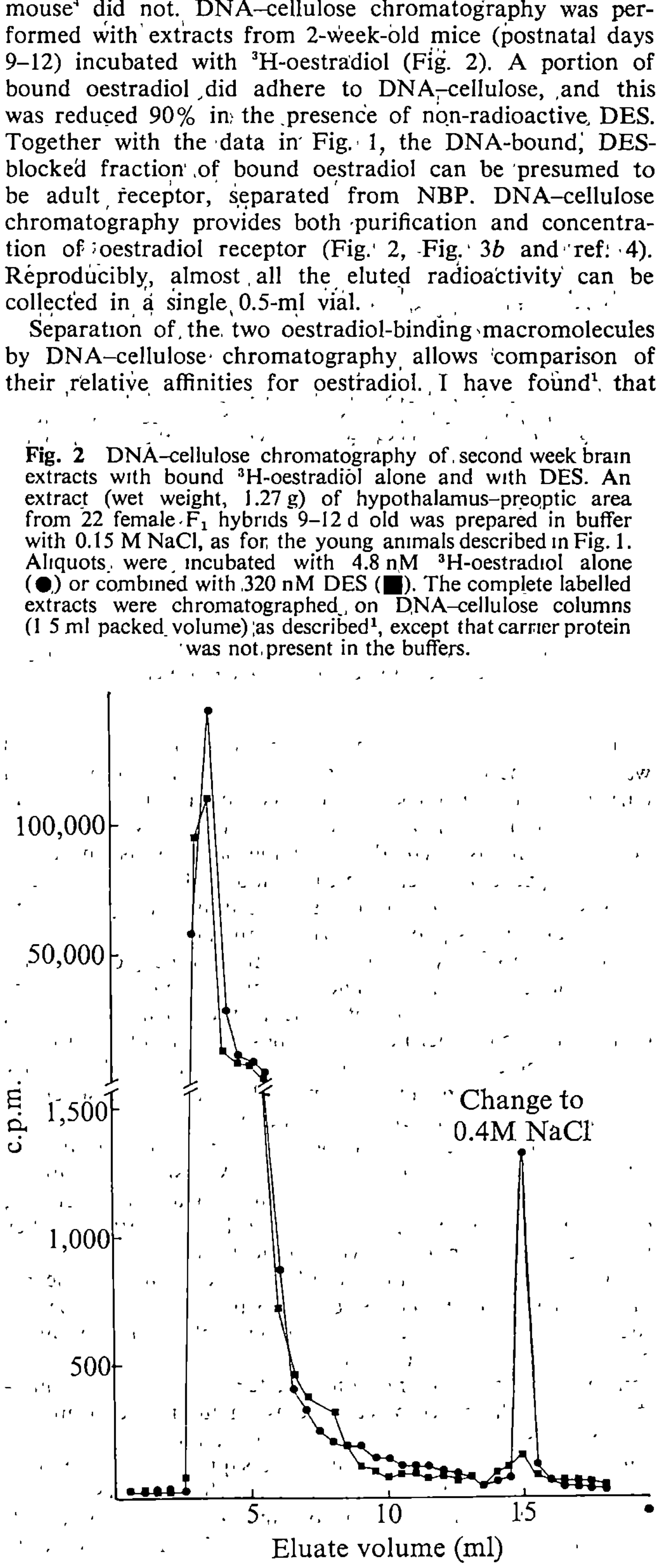
Initially oestradiol receptor was distinguished from NBP by the high affinity of the former for diethylstilboestrol (DES)[2,3] (Fig. 1) In extracts of hypothalamus–preoptic area from 4-week-old mice (postnatal days 23–24), oestradiol binding to macromolecules sedimenting at 4S and faster was decreased more than 90% by excess non-radioactive DES For 2-week-old mice (postnatal days 9–10), total oestradiol binding was higher, but was decreased only 20% by DES. As reported before[1,4], and usually seen for uterine receptor[4], prepubertal brain receptor and brain NBP both sediment (Fig 1) at about 4S in sucrose gradients containing 0.15 M NaCl, which was present to permit subsenquent DNA–cellulose chromatography. Without NaCl (not shown), prepubertal receptor and NBP have different sedimentation rates[1,4]; receptor sediments at 5.0–7.5S but NBP always has a sharp 4S sedimentation rate.

I do not believe that blood contamination accounts for the detected low affinity oestradiol binding (NBP). Preliminary experiments indicated that saline perfusion did not decrease detection of NBP, and blood pigment was not detected visually in extracts of hypothalamus–preoptic area from young mice. As suggested for young rats[3], however, NBP may be derived from blood.

Earlier experiments with DNA–cellulose chromatography[4] showed that up to half the presumed oestradiol receptor• from prepubertal mouse hypothalamus–preoptic area bound specifically to DNA, while NBP from rat[3] and mouse[4] did not. DNA–cellulose chromatography was performed with extracts from 2-week-old mice (postnatal days 9–12) incubated with ^{3}H-oestradiol (Fig. 2). A portion of bound oestradiol did adhere to DNA–cellulose, and this was reduced 90% in the presence of non-radioactive DES. Together with the data in Fig. 1, the DNA-bound, DES-blocked fraction of bound oestradiol can be presumed to be adult receptor, separated from NBP. DNA–cellulose chromatography provides both purification and concentration of oestradiol receptor (Fig. 2, Fig. 3*b* and ref. 4). Reproducibly, almost all the eluted radioactivity can be collected in a single 0.5-ml vial.

Separation of the two oestradiol-binding macromolecules by DNA–cellulose chromatography allows comparison of their relative affinities for oestradiol. I have found[1] that

Fig. 2 DNA–cellulose chromatography of second week brain extracts with bound ^{3}H-oestradiol alone and with DES. An extract (wet weight, 1.27 g) of hypothalamus–preoptic area from 22 female F$_1$ hybrids 9–12 d old was prepared in buffer with 0.15 M NaCl, as for the young animals described in Fig. 1. Aliquots were incubated with 4.8 nM ^{3}H-oestradiol alone (●) or combined with 320 nM DES (■). The complete labelled extracts were chromatographed on DNA–cellulose columns (1.5 ml packed volume) as described[1], except that carrier protein was not present in the buffers.



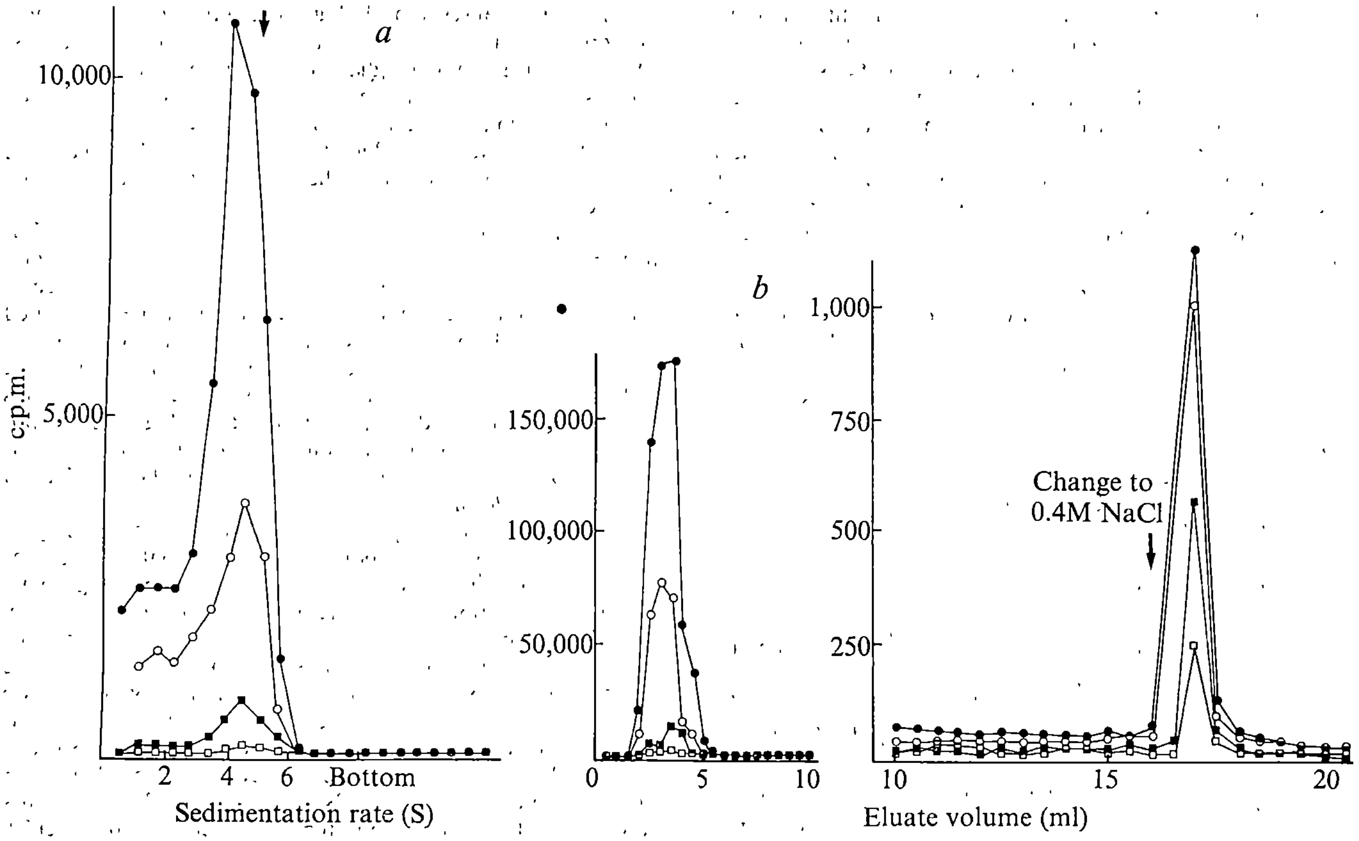


Fig. 3 Sucrose gradient sedimentation and DNA–cellulose chromatography of first week mouse brain extracts incubated with different concentrations of ^{3}H-oestradiol. Extract (wet weight, 1.93 g) was prepared with hypothalamus–preoptic area from 33 female and male F_1 hybrids 3–7 d old In an experiment comparing 2–3-d-old females and males, similar amounts of presumed oestradiol receptor were detected by DNA–cellulose chromatography, justifying the combination of females and males for this experiment. Aliquots were incubated with 1.4 nM ^{3}H-oestradiol (□); 7.2 nM ^{3}H-oestradiol (■); 36 nM ^{3}H-oestradiol (○); or 140 nM ^{3}H-oestradiol (●). Each labelled extract was freed of unbound oestradiol on a Sephadex G-25 column[1,4]. *a*, Aliquots of the Sephadex breakthrough fraction were sedimented on sucrose gradients to demonstrate that this fraction was predominantly bound to macromolecules at each concentration. The arrow indicated dansyl-bovine serum albumin (4 7S). *b*, DNA-cellulose chromatography of macromolecule-bound oestradiol. The abscissa has been split to allow two ordinates for non-DNA–cellulose-adhering NBP (eluting between 2 and 5 ml) and oestradiol receptor (eluting between 16 and 18 ml).

oestradiol receptor from prepubertal mouse hypothalamus–preoptic area is saturated by oestradiol at 4–8 nm. This is consistent with reported values[7] of 10^{-9}–10^{-10} M for the dissociation constant of adult oestradiol receptor from rat hypothalamus and related brain regions. In contrast, the dissociation constant for rat NBP is reported[3] at 1–2 × 10^{-8} M. Since I have found (not shown) that NBP, present in large amounts in young mice, binds most of the oestradiol in solution in the range 1–140 nM, oestradiol receptor in its presence should have an apparent dissociation constant higher than normal. Accordingly, the DNA-adherent portion of bound oestradiol from young animals (as Fig 2) should saturate at a higher concentration than usual if it is truly oestradiol receptor.

Oestradiol binding to oestradiol receptor and to NBP was compared for 1-week-old mice (postnatal days 3–7) after incubation with several concentrations of oestradiol (Fig. 3*b*) At higher oestradiol concentrations, total binding to NBP was greater (Fig. 3*a* and left portion in Fig. 3*b*), approaching saturation at 140 nM Oestradiol binding to presumed receptor, however, increased only about threefold between 7.2 nM and 140 nM, nearly a twentyfold concentration range. Therefore, saturation of receptor was appreciably lower than for NBP, but 2–4 times greater than observed in pre-pubertal extracts. In the same experiment performed with 4-week-old mice (not shown), maximal DNA-binding was attained at 6 nM oestradiol. With 2-d-old mice (not shown), the same qualitative results were obtained, except with more NBP than in 1 and 2-week-old animals. Thus young mouse brain contains an appreciable amount of oestradiol receptor, but its quantitative detection requires larger concentrations of ^{3}H-oestradiol than required for extracts of older animals.

Previously, receptor proteins for oestradiol have been found in the hypothalamus from adult and prepubertal rats[2-3,5-6] and mice[1,4]. Testosterone-binding proteins in rats[7-10] and mice[1] have also been reported. Autoradiographic studies with 2-d-old rats indicate accumulation of ^{3}H-oestradiol and ^{3}H-testosterone by specific regions of brain[11]. Quantitative binding studies with brain extracts from 5-d-old rats have suggested the presence of high affinity oestradiol-binding proteins[12], although regional specificity was not observed, and a small amount of presumed oestradiol receptor has been detected by sucrose gradient sedimentation of ^{3}H-oestradiol-labelled extracts from 7-d-old rats[6].

The experiments described here show that extracts of hypothalamus–preoptic area from neonatal to 2-week-old mice contain macromolecules that bind oestradiol with high affinity and that can be distinguished from NBP by their affinity for DNA and their sensitivity to DES. These properties suggest that these macromolecules are adult-like oestradiol receptors. They are present in mice less than 1 week old, within the critical period, 7–10 d after birth, when oestrogens and androgens administered to mice or rats can alter sexual differentiation permanently[13,14]. High affinity testosterone binding, in addition to oestradiol binding, has been detected in hypothalamus–preoptic area of 2-d-old mice[1,15].

The observation that NBP competes with presumed receptor for oestradiol supports the suggestion[3] that this protein may be present in neonatal brain to protect it from high levels of maternal oestrogen They might otherwise oestrogenise all young mice and rats, causing females to be sterile[13]. DNA–cellulose separation methods make it possible to test this hypothesis quantitatively. Finally, these results elicit the caution that at least three factors must be included in equations of oestrogen reception in young rodent brain: oestrogen levels, receptor levels and levels of competing binding macromolecules In other receptor systems, specific competing macromolecules might also need to be considered.

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Distinction between nerve growth factor and glial factor

THE morphogenesis of individual cell types can be studied in tissue culture: for example neuroblastoma cells can be induced to differentiate by a macromolecular factor released by cultured glioma cells[1]. On the other hand, survival and process formation in cell cultures of dissociated ganglia from embryonic chicks, newborn mice or rats require the presence of nerve growth factor[2,6]. An enriched population of non-neuronal cells can replace NGF in these systems and their effect can be impaired by addition of anti-NGF antibody[2,3]. The fact that NGF has been detected in the homogenate of solid tumours induced by subcutaneous injection of glioma cells previously grown in culture, and that a partially purified fraction of this homogenate induces morphological differentiation in neuroblastoma cells[4] suggests that NGF and glial factor are the same molecule. We present evidence that NGF and glial factor are molecules with different properties and distinct specificities.

The respective biological activities of NGF and glial factor have been investigated in cultures of chick dorsal root ganglia[5], in dissociated cells cultured from rat superior cervical ganglia[6] and in mouse neuroblastoma cell cultures[1]. We confirmed that 2.5S NGF induces process formation in cultures of chick dorsal root ganglia[5]. In this system, NGF 0.004 μg ml^{-1} and 0.016 μg ml^{-1} gave responses + and ++++, respectively (responses defined by Fenton[8]). In dissociated cells cultured from the rat superior cervical ganglia[6] 0.125 μg ml^{-1} was the lowest concentration at which an effect of 2.5S NGF could be detected, and 1 μg ml^{-1} was the concentration routinely used. We found, however, that 2.5S NGF , tested at concentrations of 0.05, 0.1, 0.5, 1.0 and 10 μg ml^{-1}, does not induce morphological differentiation in neuroblastoma cells. In contrast the glial factor, which causes morphological differentiation of neuroblastoma cells, had no effect in the two other biological assays. The different amounts of glia-conditioned medium or concentrated serum-free medium[1] tested in these bioassays represented 0.5, 1 or 10 times the glial factor activity necessary to obtain optimal morphological differentiation of neuroblastoma cells. These results indicate that the two factors have distinct specificities.

We further distinguished the factors by immunological methods. Purified anti-NGF antibody (K. S., C. Gagnon and H. T., in preparation), able to prevent NGF-induced process formation in the chick dorsal root ganglia and in the dissociated cell cultures of the rat superior cervical ganglia, does not interfere with the effect of glial factor in the neuroblastoma cell system. Table 1 shows that different concentrations of this purified antibody could not inhibit the morphological differentiation induced by the glia-conditioned medium. The lack of immunological cross reactivity indicates that the two factors are distinct molecules.

Finally, NGF and a partially purified preparation of glial factor were compared on sodium dodecyl sulphate–polyacrylamide gel electrophoresis (Fig. 1). In the gel in which the partially purified preparation of glial factor had been electrophoresed, not even a faint trace of staining could be detected where the 2.5S NGF subunit migrates. Assuming that we could have detected 0.2 μg of NGF in this system and knowing that we applied 738 U of glial factor activity, then 0.27 ng of NGF could be maximally present per unit of glial factor. Since it takes 10 U per 5 ml of culture medium to detect glial factor activity, the concentration of NGF present would be 20 times lower than the minimal concentration detectable in the chick dorsal root ganglia assay.

We conclude that NGF and glial factor are different molecules

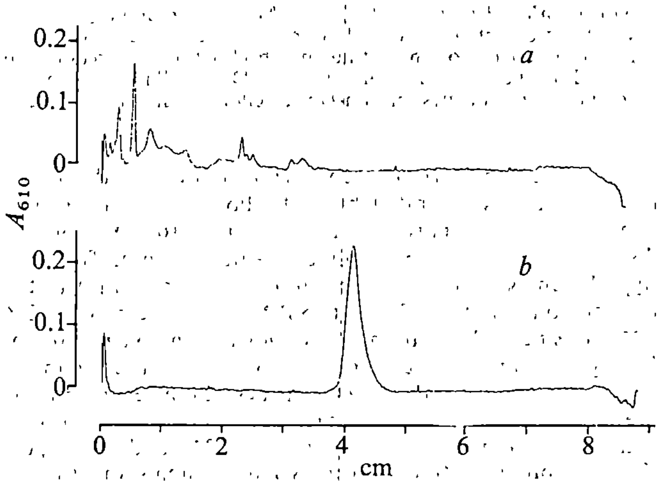

Fig. 1 Electrophoretic comparison of NGF and glial factor C-6 glial cells were grown in rollers in Dulbecco's modified Eagles' medium supplemented with 10% foetal calf serum (Colorado Serum Co) After 72 h the medium was changed and the cells were incubated for 24 h in the same medium without foetal calf serum This serum-free conditioned medium was concentrated 20-fold with a UM-10 Diaflo membrane and dialysed against phosphate buffer (6.7 mM $Na_2HPO_4 2H_2O$, 2 86 mM KH_2PO_4, *p*H 7 35). The material was then loaded on a Whatman DE 52 cellulose column equilibrated with this buffer and submitted to stepwise elution with 0.2 M and 0.6 M NaCl in the same buffer. The glial factor activity is eluted in a very sharp peak just after the switch to the buffer containing 0.6 M NaCl. The main fraction of the peak had 3 μg of protein and contained 51% of the activity loaded on the column This step of purification usually gives up to a 60-fold increase in the glial factor specific activity This fraction was dialysed against double distilled water, lyophilised and resuspended in the sample buffer of the electrophoretic system[9,10]. The 15% polyacrylamide gels were stained with 0 1% Coomassie Blue R250 in 50% methanol and 7.5% acetic acid After destaining with the same solvent mixture, they were scanned at 610 nm using a Zeiss PMQ II spectrophotometer. *a*, Partially purified glial factor; *b*, 20 μg of NGF purified by the method of Bocchini and Angeletti[11].

Table 1 Constancy of glia-induced morphological differentiation of neuroblastoma cells in the presence of different concentrations of anti-NGF antibody

Medium	μg Anti-NGF per ml	Cells with processes (%)
Conditioned	0	77.6±4.7
Conditioned	0 1	75.6±3.4
Conditioned	1.0	76.0±4.9
Conditioned	10.0	74.8±3.5
Normal	0	5.9±0.8
Normal	10.0	5.9±2.3

The induction and determination of morphological differentiation was carried out as described previously[1]. The purified anti-NGF added to these cultures completely inhibited process formation in the chick dorsal root ganglia bioassay at a concentration of 0.1 μg ml^{-1}.

with distinct specificities. The action on the morphological differentiation of neuroblastoma cells observed by Longo and Penhoet[4] with a partially purified NGF fraction from a solid tumour of glial origin could be explained by contamination with glial factor. Furthermore, as Levi-Montalcini[7] pointed out, it is not yet certain that the NGF produced in the solid tumour is of glial origin.

Since glial factor acts on primary cultures of dissociated cells from embryonic rat brain (Y. Schürch-Rathgeb, and D. M., in preparation) and not on similar cultures of the superior cervical ganglia, we propose that glial factor and NGF produce similar effects but on different target cells. Further studies are required to establish the sites of action, the specificity and the possible morphogenic implications of the glial factor in the central nervous system.

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Supercoiled DNA folded by non-histone proteins in cultured mammalian cells

A LONG-CHAIN DNA molecule must be highly folded within the nucleus of the eukaryotic cell with some regularity. Information about the structure of chromatin has come from X-ray diffraction[1–4], physicochemical studies[5,6], electron microscopy[7–11] and nuclease digestion[12–15], and suggested that chromatin from eukaryotes comprises a linear array of spherical particles in which DNA is supercoiled around a core of oligomers of histones[16,17]. We have used another approach to assess the structure of DNA in the nucleus of cultured mammalian cells, and have evidence suggesting that when all the histones and most of the non-histone proteins are removed from chromatin, DNA is supercoiled and folded by a few non-histone proteins tightly bound to it.

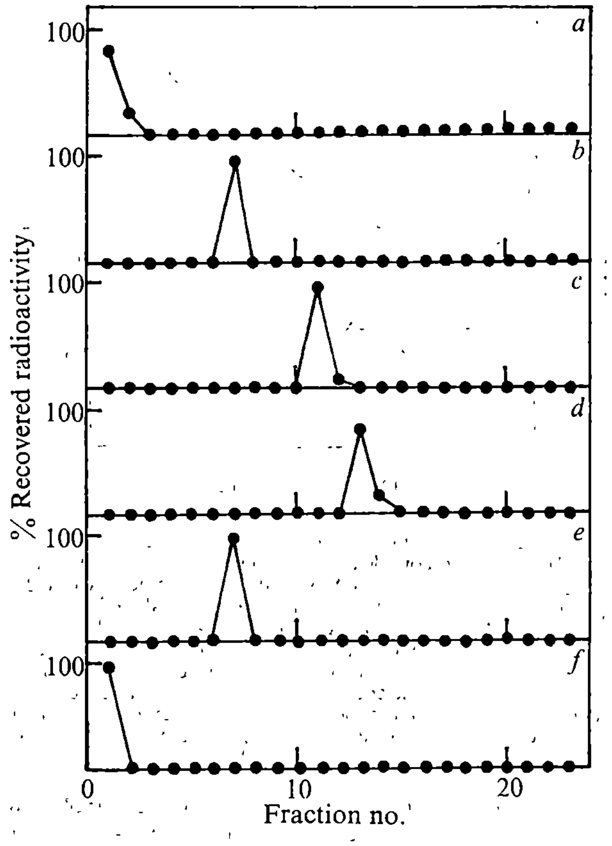


Fig. 1 Effect of actinomycin D on the sedimentation of a DNA–protein complex. FM3A cells established from mouse mammary carcinoma[23] were cultured in suspension as before[18]. Cells labelled with ^{14}C-thymidine (62 mCi mmol^{-1}) at 0.05 μCi ml^{-1} for 24 h were treated at a density of 3×10^5 cells per ml with actinomycin D (Merck, Sharp and Dohme) for 30 min at 37 °C at various concentrations (*a*, 0; *b*, 1; *c*, 3; *d*, 10; *e*, 70; *f*, 100 μg ml^{-1}). DNA–protein complex released by lysing the cells in 1.3% SDS solution on top of a neutral sucrose gradient was spun at 36,000*g* for 60 min at 20 °C (ref. 18). After centrifugation, fractions were collected from the bottom of the tube and acid-precipitable radioactivity was counted in Beckman scintillation spectrophotometer.

When FM3A cells labelled with ^{14}C-thymidine were analysed by sodium dodecyl sulphate (SDS)–neutral sucrose gradient centrifugation, DNA sedimented as a complex with proteins containing disulphides[18–20]. When cells were treated with various concentrations of actinomycin D and analysed in the same way, the sedimentation rate of the complex varied depending on the concentration of the agent used (Figs 1 and 2). As Fig. 2 shows, the amount of actinomycin D bound to DNA increased proportionately as the concentration of the drug added to the culture medium was increased up to 70 μg ml^{-1}. The minimum sedimentation rate of the complex was obtained at a concentration of about 20 μg ml^{-1}. Above or below this concentration sedimentation rates were larger. The sedimentation rate of the complex from untreated control cells was reduced if it was treated with Pronase known to be free of DNase activity in the top layer of the gradient[18,19] (Fig. 2). Trypsin and 2-mercaptoethanol caused the same reduction in the sedimentation rate of the complex as did Pronase[19,20], while RNase A and T1 and phospholipase A and C had no effect (ref. 19 and our unpublished data). The Pronase-digested DNA–protein complex from actinomycin-treated cells showed a similar but less pronounced transition in the sedimentation rate with a minimum value at 20 μg ml^{-1}. When cells were irradiated with γ rays before treatment with actinomycin

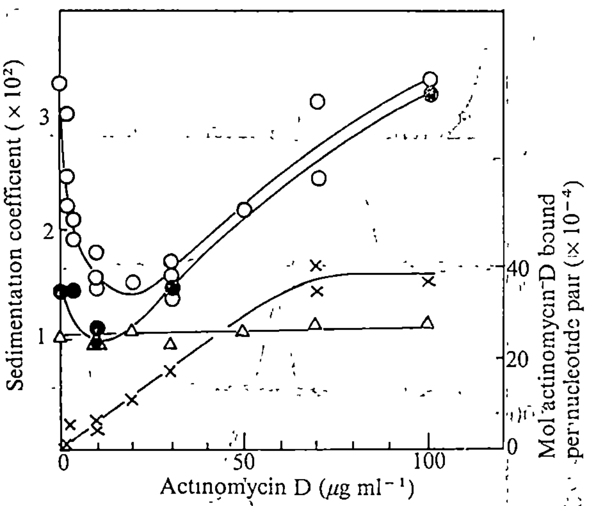


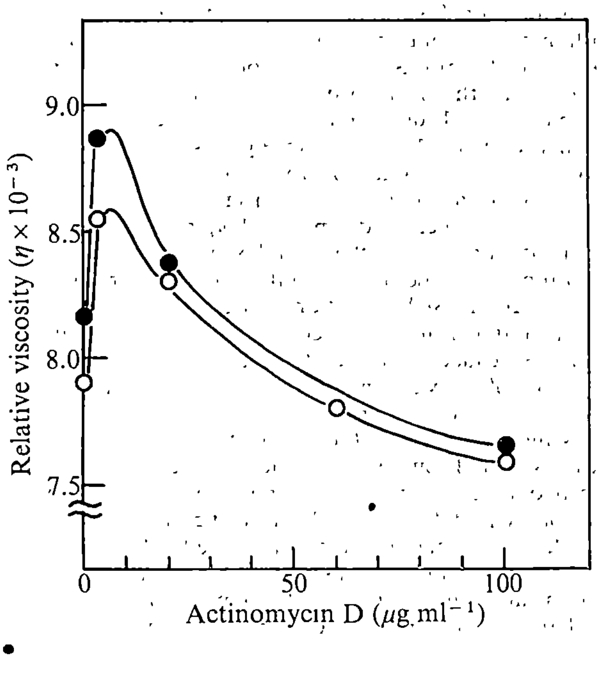
Fig. 2 Effect of actinomycin D and Pronase on the sedimentation of DNA–protein complex. FM3A cells were treated with actinomycin D and lysed in SDS on top of the gradient with (●) or without (○) Pronase E (1.5 mg ml^{-1}). Cells irradiated with γ rays at a dose of 24,000 r from a ^{60}Co source and treated with actinomycin D (△). Sedimentation coefficient was calculated with ^{3}H-λ_{c1} particles as a reference marker. The amount of actinomycin D bound per nucleotide pair of DNA calculated using ^{3}H-actinomycin D bound in the DNA fraction was the same whether cells were irradiated or not, and so those from control cells were plotted (×).

D the characteristic biphasic transition was abolished.

The viscometric behaviour of the DNA–protein complex was the opposite to that in sedimentation analysis (Fig. 3). The relative viscosity of the complex increased initially to a maximum and then decreased through continued binding of actinomycin D. Relative viscosity increased when the complex was treated with Pronase.

A large quantity of the DNA–protein complex was collected from scaled up gradients, and the proteins contained in it were dissociated from DNA with 2-mercaptoethanol and analysed by SDS–polyacrylamide gel electrophoresis. As Fig. 4 shows, two non-histone proteins with apparent molecular weights of approximately 70,000 and 55,000 were obtained.

Fig. 3 Effect of actinomycin D and Pronase on the viscosity of the DNA–protein complex. FM3A cells (5×10^5 cells per ml) were treated with various concentrations of actinomycin D for 30 min at 37 °C and lysed by mixing with the same volume of 2% SDS with (●) or without (○) Pronase E (1.5 mg ml^{-1}) in the chamber of a capillary type Ostwald viscometer. Relative viscosity was calculated with water as a reference standard.



Taken together our experiments suggested a basic structure of DNA in the nucleus of the mammalian cell. (1) DNA is supercoiled and folded by a few non-histone proteins. (2) Dissociation of the proteins from DNA releases folds in DNA, but the DNA remains supercoiled and thus folds and supercoils maintain the compactness of the DNA–protein complex which has a large sedimentation rate and a small relative viscosity. (3) Unwinding of supercoils by intercalating agents and/or unfolding after the disruption of protein cores converts the DNA into a relaxed structure with a small sedimentation rate and a large relative viscosity. The minimum sedimentation rate and the maximum relative viscosity in folded and unfolded DNA are obtained when all superhelical turns are lost. Continued binding of the intercalating agents results in the formation of superhelical turns of opposite handedness, and thus an increase in sedimentation rate and a decrease in relative viscosity. Further support for this contention comes from two sources. First, results similar to those described here with actinomycin D were obtained with other intercalating agents such as acriflavin and ethidium bromide. Second, in a microscopic autoradiogram of the DNA–protein complex spread on a Millipore membrane, the slow sedimenting complex was shown to be a relaxed mass of DNA fibres in contrast to a compact mass of DNA from the fast sedimenting complex.

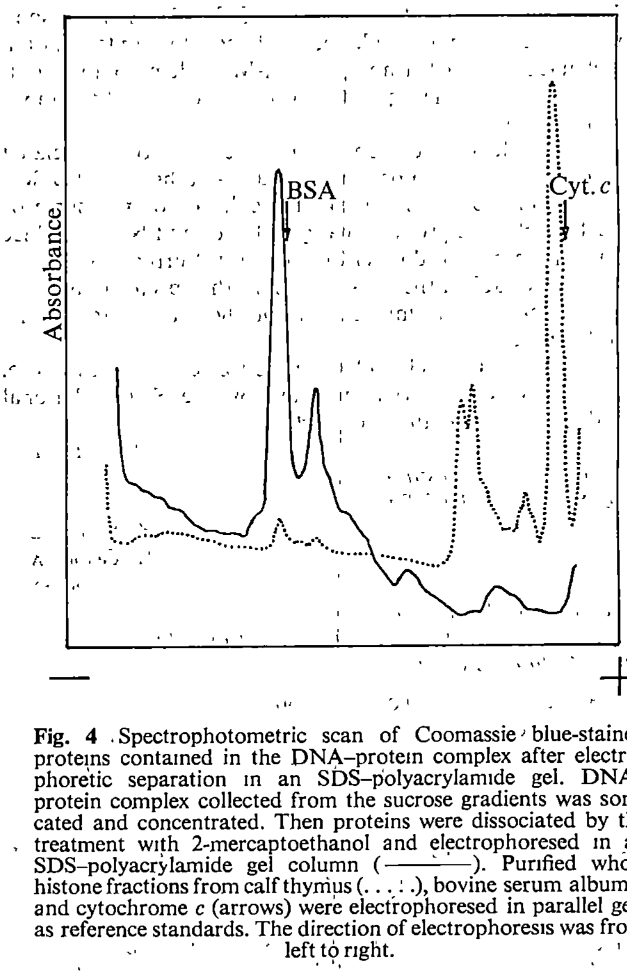


Fig. 4 Spectrophotometric scan of Coomassie blue-stained proteins contained in the DNA–protein complex after electrophoretic separation in an SDS–polyacrylamide gel. DNA–protein complex collected from the sucrose gradients was sonicated and concentrated. Then proteins were dissociated by the treatment with 2-mercaptoethanol and electrophoresed in an SDS–polyacrylamide gel column (———). Purified whole histone fractions from calf thymus (.....), bovine serum albumin and cytochrome *c* (arrows) were electrophoresed in parallel gels as reference standards. The direction of electrophoresis was from left to right.

The supercoiled structure of DNA suggested here is for the DNA–protein complex obtained by dissociation of all the histones and most of the non-histone proteins from chromatin, and thus may have derived from the supercoils localised in the unit spherical particles of chromatin. A similar structural model of DNA in higher organisms has been proposed[21,22] in which DNA is folded into loops held by some proteins.

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Nucleosome formation abolishes base-specific binding of histones

CHROMATIN has a subunit structure[1–5] and each subunit, for which the term nucleosome has been suggested[5], has been proposed to consist of two of each of the four histones H2A, H2B, H3 and H4, and 200 base pairs of DNA (ref. 3). Do the histones responsible for generating the nucleosomes bind to specific sequences of the DNA? Heterologous reconstitution experiments showed that the four histones bound to DNA from bacteria or viruses gave rise to the same X-ray diffraction pattern[6], the same size of nuclease-resistant DNA fragments[7], and the same subunit structure, shown by electron microscopy[5], as obtained for native chromatin. These findings suggest that the nucleosomes do not contain specific DNA sequences. This agrees with the apparently random distribution of nucleosomes along adenovirus DNA[5]. On the other hand, there is evidence that individual histones and polypeptides like polylysine or polyarginine bind preferentially to certain DNA sequences[8–10].

We have approached the question of the specificity of binding of histones to DNA sequences in the following way: λ*dv*21 DNA, a small plasmid of molecular weight 2.1×10^6, was linearised by digestion with restriction nuclease *Hin*dIII, and λ*dv*21 histone complexes were reconstituted with calf thymus histones. The location of histones on the linear DNA was then investigated using restriction nuclease *Hin*dII which cleaves λ*dv*21 DNA at five sites (Fig. 1). The individual histones as well as the tetramer complex $(H3)_2$ $(H4)_2$ (refs 12, 13) all protected two adjacent *Hin*dII sites from cleavage much better than the other three, indicating specificity of binding. This specificity was not found when complexes reconstituted with the four histones which constitute the nucleosome (that is H2A, H2B, H3 and H4) were cleaved with the restriction enzyme.

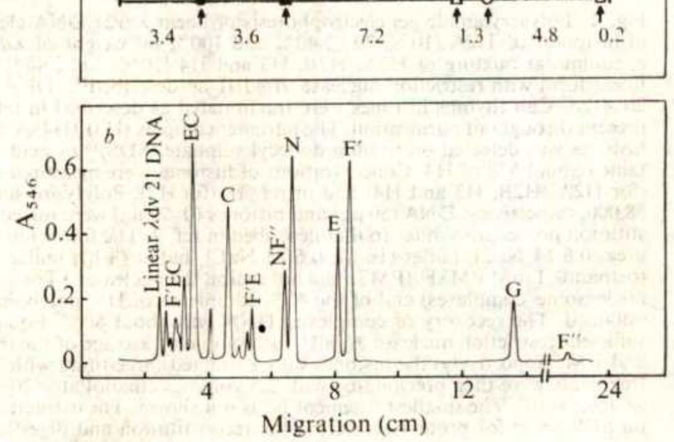
Figure 2 shows *Hin*dII digestion patterns of complexes of linear λ*dv*21 DNA with increasing amounts of histones. Some λ*dv*21 cleavage sites were protected by histones, for partial digestion fragments appeared in conditions just sufficient for complete cleavage of the free DNA. In more extensive digestion conditions complete cleavage of the complexes could be achieved (not shown). Thus the protection of cleavage sites by histones can be overcome by restriction nuclease. To evaluate the protection of cleavage sites quantitatively, we used densitometry of polyacrylamide gels. A densitogram of a partial digest of λ*dv*21 DNA is shown in Fig. 1*b*. As Fig. 3 shows, all the histones, when tested individually, protected the two cleavage sites on the left of the map (between F′, E, and C) better than the three other sites. In the 100% H2A complex the fragments C, E and F′ were missing and fragment F′EC appeared instead. A similar specificity was found with H1 and H2B and to a lesser extent with H3 and H4.

The extent of protection differed for the individual histones. H1 protected all five cleavage sites very efficiently. Polylysine also protected the DNA against the nuclease quite efficiently, but it did not show the same specificity as did the histones. Instead, it had slight preference for the left cleavage site between E and C and the right one between N and F″. Polyarginine behaved much like polylysine (not shown).

Preferential protection by histones is not unique to cleavage sites for *Hin*dII. We also found preferential protection of cleavage sites for *Bacillus subtilis* restriction nuclease which were close to the *Hin*dII cleavage site between F′ and E (Fig. 1). The preferential protection of the left-hand *Hin*dII cleavage sites may be due to preferential binding of the individual histones to particular DNA sequences at or near the cleavage sites. The protected *Hin*dII site between F′ and E lies in the rightward operator and promoter region of phage λ, which has been sequenced and found to consist of alternative highly AT-rich and highly GC-rich DNA sequences, each about twelve nucleotides long[20]. This microheterogeneity might favour preferential binding of individual histones to this region.

It should be noted that the specificity of histone binding

Fig. 1 *Hin*dII digestion of linear λ*dv*21 DNA. *a*, Cleavage map of linear λ*dv*21 DNA. The *Hin*dIII site is located in fragment F, giving rise to fragments F′ and F″. Molecular weights ($\times 10^{-5}$), of the fragments are indicated[11]. *b*, Densitogram of a gel electrophoretic separation of fragments from a partial *Hin*dII digestion of linear λ*dv*21 DNA. For experimental details see legend of Fig. 2. The assignment of bands to particular *Hin*dII fragments was based on the determination of molecular weights which were compared with those calculated from the cleavage map. Some fragments are indicated.



depended on the salt concentration. When histone DNA complexes were digested which had been dialysed against 100 mM NaCl in buffer in the last reconstruction step, no specificity was observed.

The tetramer complexe $(H3)_2(H4)_2$ which was postulated[3] to be the core of the nucleosomes clearly showed the same specificity of protection as the individual histones (Figs 2*b* and 3). Protection of the two adjacent cleavage sites by the tetramer complex was more specific than that by H3 and H4 tested individually. The specificity of protection was lost, however, when λ*dv*21 DNA was complexed with an equimolar mixture of the four histones which constitute the nucleosome: all five cleavage sites were protected to the same extent (Fig 3). The very slight specificity of protection observed in the 40% complex may be due to incomplete nucleosome formation. Fragment patterns very similar to those of Fig. 2*c* could also be produced by partial digestion of histone-free linear λ*dv*21 DNA (for example Fig. 1*b*) while those of Fig. 2*a* and *b* were unique to the histone complexes. This also demonstrated that the protection of cleavage sites in the presence of the four nucleosome-forming histones was unspecific. When an equimolar amount of H1, in addition to the four histones, was included in the reconstitution mixture, the protection of all five cleavage sites was more efficient than without H1, but again there was preferential protection of the two cleavage sites on the left side of the map (Fig. 3).

To determine whether nucleosomes had been formed in our reconstitution procedures we did digestion experiments with micrococcal nuclease. Degradation of the complexes between DNA and the four histones gave rise to discrete DNA fragments of about 180 and 360 base pairs, similar in size to those obtained from native chromatin[4]. When reconstitution was carried out in the absence of urea[12,21,22], DNA fragments of threefold unit length were also detected on digestion with micrococcal nuclease. More than 80% of the DNA could be converted into the monomer band when the 100% complex which had been formed in the absence

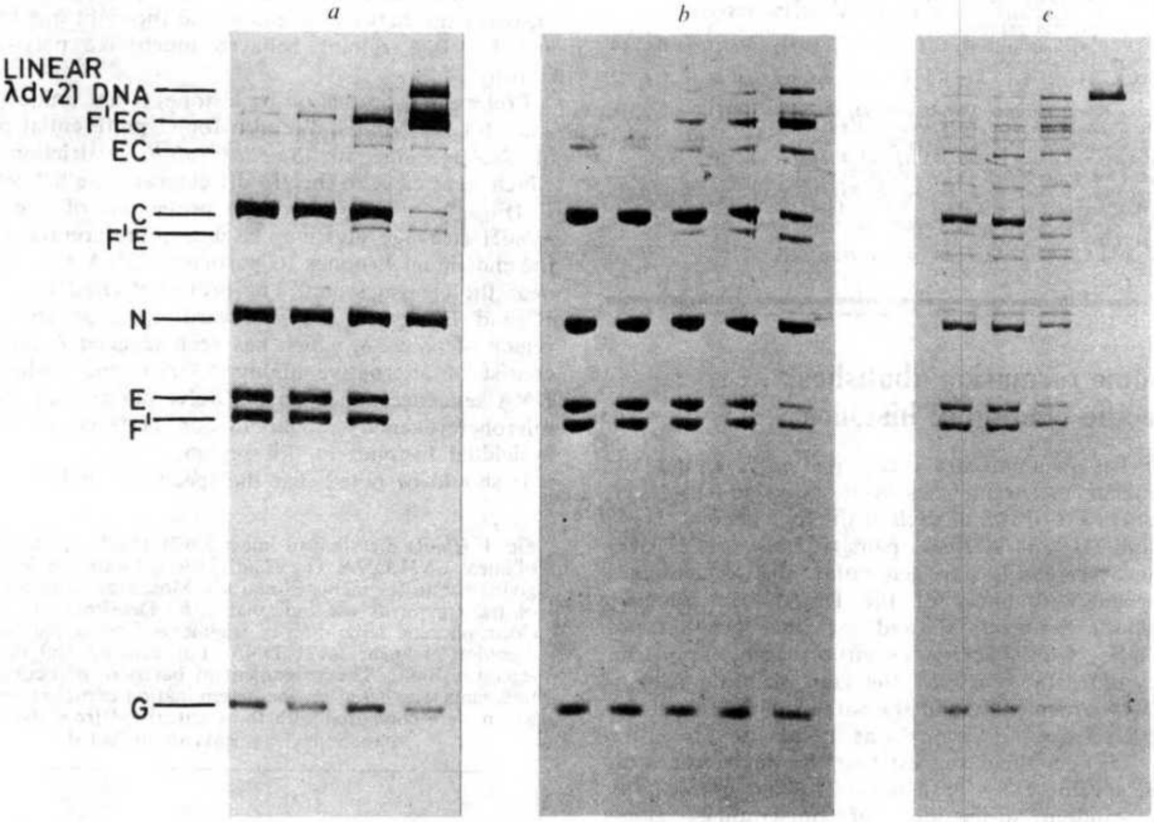


Fig. 2 Polyacrylamide gel electrophoresis of linear λ*dv*21 DNA cleaved with *Hin*dII after complex formation with increasing amounts of histones. *a*, H2A (10%, 20%, 40% and 100% by weight of λ*dv*21 DNA); *b*, $(H3)_2(H4)_2$ tetramer (0%, 5%, 20%, 40% and 100%); *c*, equimolar mixture of H2A, H2B, H3 and H4 (10%, 20%, 40% and 100%). λ*dv*21 plasmid DNA was isolated and converted to the linear form with restriction nuclease *Hin*dIII as described[11]. DNA concentrations were determined spectrophotometrically (1mg ml^{-1} = 20 A_{260}). Calf thymus histones were fractionated as described in ref. 14. except that 1 mM phenylmethyl sulphonyl fluoride (PMSF) was present throughout purification. The tetramer complex $(H3)_2(H4)_2$ (ref. 12) was purified as described[13]. No cross contamination of individual histones was detected on sodium dodecyl sulphate (SDS)[15] or acid–urea gels[16] (3–5% would have been seen), except for H2B which contained about 5% of H4. Concentrations of histones were measured spectrophotometrically using the extinction coefficients given in ref. 17 (for H2A, H2B, H3 and H4) and in ref. 18 (for H1). Polylysine and polyarginine (Sigma) had average molecular weights of 30,000 and 58,000, respectively. DNA (50 µg) and histones (0–50 µg) were mixed in small dialysis bags in a total of 0.35 ml and taken through a reconstitution procedure similar to that described in ref. 7. The following dialysis steps were carried out: 5 M urea, 3 M NaCl, buffer (8 h); 5 M urea, 0.6 M NaCl, buffer (16 h); 0.6 M NaCl, buffer (8 h); buffer (16 h). The buffer used was 10 mM Tris-HCl, *p*H 8.0, 1 mM mercaptoethanol, 1 mM PMSF .(PMSF did not inhibit the nucleases.) The solution of the 100% complexes (except for the H2A–, tetramer–, and the nucleosome–complexes) and of the 40% complexes of H1, polylysine,and polyarginine were turbid; in all other cases clear solutions were obtained. The recovery of complexed DNA was about 80%. Equal amounts of the complexes were digested for 1 h at 37 °C with just sufficient restriction nuclease *Hin*dII for complete cleavage of the free DNA. After addition of SDS and $NaClO_4$ (final concentrations 1% and 1 M, respectively) the histones were extracted three times with 2 volumes of phenol–chloroform–isoamyl alcohol (24:24:1); the DNA fragments were then precipitated with 2.5 volumes ethanol at −20 °C overnight, and analysed on 4% polyacrylamide slab gels essentially as described[19]. The smallest fragment F″ is not shown. The extracted histones were dialysed against 1 mM PMSF, lyophilised and analysed on SDS gels[15] for proteolysis during the reconstitution and digestion procedures. No degradation was observed. The letters used for the indicated fragments are those of Fig. 1.

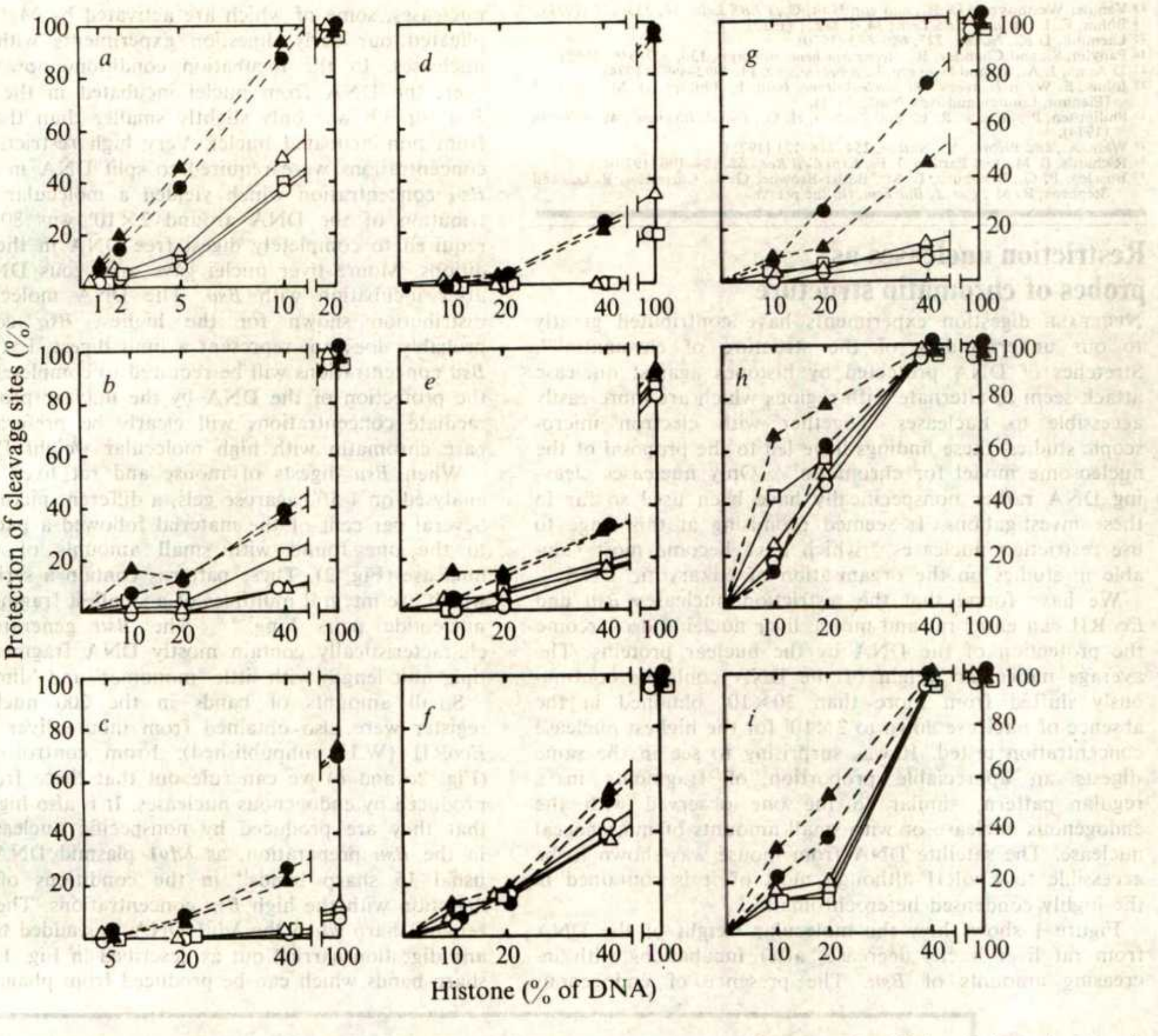


Fig. 3 Quantitative analysis of protection of *Hin*dII cleavage sites in linear λ*dv*21 DNA by histones and polylysine. Polyacrylamide gel electrophoretic separations of DNA fragments of the *Hin*dII-cleaved complexes were stained (Fig. 2), scanned and the peak areas of the densitograms (Fig. 1*b*) were measured planimetrically[19]. The extent of protection of a particular cleavage site was calculated from the relative amounts of the fragments in the digest. From the molecular weights of the fragments their molar amounts were obtained. Based on these data, the extent (on a molar basis) to which each of the cleavage sites was protected was calculated. Symbols used for the cleavage sites are those of Fig. 1*a*. *a*, H1; *b*, H3; *c*, $(H3)_2(H4)_2$; *d*, H2A; *e*, H4; *f*, H2A+H2B+H3+H4; *g*, H2B; *h*, polylysine; *i*, H1+H2A+H2B+H3+H4.

of urea was digested with micrococcal nuclease. No discrete DNA fragments were found when complexes of DNA with the histone pairs H2A+H2B or H3+H4 were digested. This agrees with the observation that all four histones are required to reconstruct the subunit structure[5,22]. It should be mentioned that in the complexes prepared in the absence of urea, the preferential protection of the two left-hand *Hin*dII cleavage sites by individual histones (H1, H2A, and the tetramer $(H3)_2(H4)_2$ were tested) was maintained and the unspecific protection by nucleosomes was unchanged.

The individual histones seem to be located mainly at or near the preferentially protected cleavage sites. It is also conceivable that they are randomly distributed but bind in some way specifically to those sites. The absence of specificity of protection by nucleosomes indicates that they are distributed randomly along the DNA. We conclude that interactions between the four histones leading to nucleosome formation change the binding behaviour of the histones to DNA in a pronounced way. H1 when present in addition to the nucleosome-forming histones may interact separately with the DNA, either between the nucleosomes or on the outside of the nucleosomes, thus giving rise to a specificity of protection superimposed on the unspecific protection by the nucleosomes.

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Restriction nucleases as probes of chromatin structure

NUCLEASE digestion experiments have contributed greatly to our understanding of the structure of chromatin[1–4]. Stretches of DNA protected by histones against nuclease attack seem to alternate with regions which are more easily accessible to nucleases. Together with electron microscopic studies, these findings have led to the proposal of the nucleosome model for chromatin[5–7]. Only nucleases cleaving DNA rather nonspecifically have been used so far in these investigations. It seemed promising at this stage to use restriction nucleases[8,9] which have become most valuable in studies on the organisation of eukaryotic DNA.

We have found that the restriction nucleases *Bsu* and *Eco*RII can enter rat and mouse liver nuclei and overcome the protection of the DNA by the nuclear proteins. The average molecular weight of the DNA could be continuously shifted from more than 30×10^6 obtained in the absence of nuclease down to 2×10^6 for the highest nuclease concentration tested. It was surprising to see in the same digests an appreciable proportion of fragments in a regular pattern, similar to the one observed with the endogenous nuclease or with small amounts of micrococcal nuclease. The satellite DNA from mouse was shown to be accessible to *Eco*RII although most of it is contained in the highly condensed heterochromatin[10].

Figure 1 shows how the molecular weight of the DNA from rat liver nuclei decreases after incubations with increasing amounts of *Bsu*. The presence of endogenous nucleases, some of which are activated by Mg^{2+}, had complicated our early digestion experiments with restriction nucleases. In the incubation conditions now used, however, the DNA from nuclei incubated in the absence of *Bsu* for 3 h was only slightly smaller than that extracted from non-incubated nuclei. Very high restriction nuclease concentrations were required to split DNA in nuclei. The *Bsu* concentration which yielded a molecular weight distribution of the DNA around 2×10^6 was 80 times that required to completely digest free DNA in the same conditions. Mouse liver nuclei gave analogous DNA patterns after incubation with *Bsu*. The DNA molecular weight distribution shown for the highest *Bsu* concentration probably does not represent a limit digest. Extremely high *Bsu* concentrations will be required to completely overcome the protection of the DNA by the nuclear proteins. Intermediate concentrations will clearly be preferable to prepare chromatin with high molecular weight DNA.

When *Bsu* digests of mouse and rat liver nuclei were analysed on 1.5% agarose gels, a different picture emerged. Several per cent of the material followed a pattern similar to the one found with small amounts of micrococcal nuclease (Fig. 2). These patterns contain a series of bands which are integral multiples of a smallest fragment 180–200 nucleotide pairs long[2–4,6]. The *Bsu* generated patterns characteristically contain mostly DNA fragments of multiple unit length with little 'monomer' and 'dimer' present.

Small amounts of bands in the 200 nucleotide pair register were also obtained from mouse liver nuclei with *Eco*RII (W.H., unpublished). From control experiments (Fig. 2*a* and *b*) we can rule out that these fragments are produced by endogenous nucleases. It is also highly unlikely that they are produced by nonspecific nuclease activities in the *Bsu* preparation, as λ*dv*1 plasmid DNA yields the usual 15 sharp bands[14] in the conditions of chromatin digestion with the high *Bsu* concentrations. The bands also remain sharp when the λ*dv*1 DNA was added to chromatin and digestion carried out as described in Fig. 1. Additional sharp bands which can be produced from phage DNA with

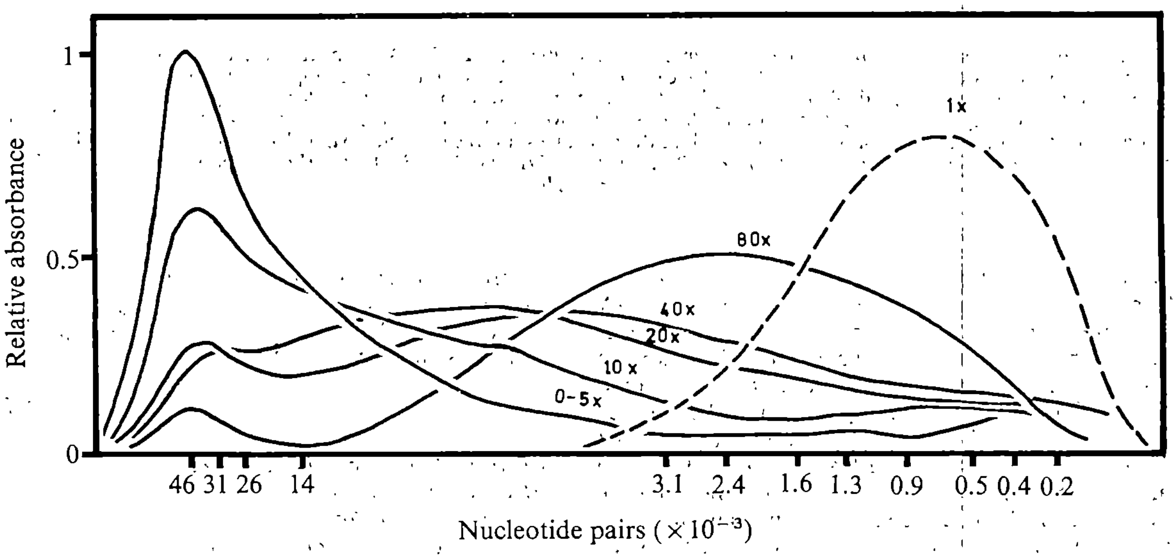

Fig. 1 Digestion of DNA in rat liver nuclei with *Bsu* analysed on 0.3% agarose gels. Nuclei were prepared as described previously[1] and used directly or frozen as a pellet in liquid nitrogen and resuspended by gentle homogenisation before use. Aliquots containing 0.5 A_{260} units DNA were incubated in 60 mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 0.2 mM EDTA, 0.2 mM EGTA, 15 mM β-mercaptoethanol, 15 mM Tris-HCl, *p*H 7.4, 10 mM MgCl$_2$ with different *Bsu* concentrations. Incubations were for 3 h at 37 °C in a total volume of 50 μl. *Bsu* was prepared as described previously[11]. The incubations were terminated by the addition of EDTA, sodium perchlorate, and SDS to give final concentrations of 50 mM, 1 M and 1%, respectively; DNA was extracted twice with phenol, once with chloroform–isoamylalcohol (24:1, v/v), precipitated with 2 volumes ethanol, and dissolved in 10 mM Tris-HCl, *p*H 7.4, 1 mM EDTA. 0.05 A_{260} units of DNA were applied to a 0.3% horizontal agarose gel, run in the Tris-borate buffer system of [12] including 1 μg ml^{-1} ethidium bromide. Time of electrophoresis was 36 h at 20 V. T5 DNA digested with *Eco*RI[13] and λ*dv*1 plasmid DNA digested with *Bsu*[14] served as molecular weight markers. At the end of the run the gel was photographed in ultraviolet light (Osram HQV lamps) with a Durst Laborator 138S equipped with an orange filter (Schott OG570). A graphical film (Agfa P23p, 13×18 cm) was used. The negatives were scanned and the peak areas normalised. Peak areas were shown to be directly proportional to the amounts of DNA over at least a factor of 10. The numbers refer to the concentrations of *Bsu* used. 1×*Bsu* is the concentration required to completely degrade free DNA in 3 h in these conditions. - - -, Complete digest of free DNA with *Bsu*; ———, DNA from rat liver nuclei digested with increasing concentrations of *Bsu*.

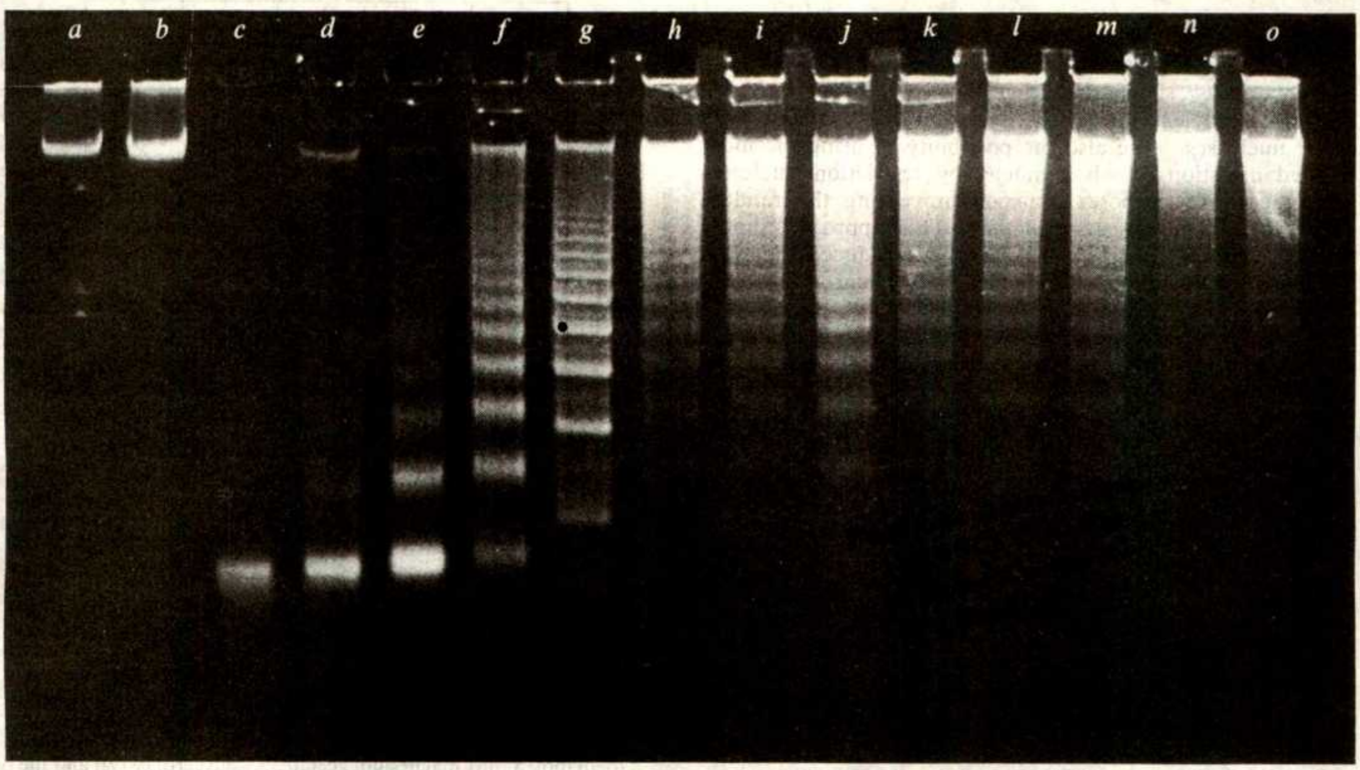

Fig. 2 Digestion of DNA in mouse liver nuclei with micrococcal nuclease and *Bsu* analysed on 1.5% agarose gels. Mouse liver nuclei were incubated in the absence or presence of nuclease for different periods of time, and the DNA extracted and analysed as described in Fig. 1 except that a 1.5% vertical agarose slab gel was used. When micrococcal nuclease was used, $MgCl_2$ in the incubation medium was replaced by 1 mM $CaCl_2$. DNA samples from nuclei digested with micrococcal nuclease (Worthington) are in slots *c–f* (1 h; 30, 10, 3, 1 U ml^{-1}) and with *Bsu* in slots *h–o*. *h–j*, 100 × *Bsu* (1 h, 2 h and 3 h); *k–m*, 40 × *Bsu* (1 h, 2 h, and 3 h); *n–o*, 20 × *Bsu* (1 h and 3 h). 1 × *Bsu* was the concentration required to completely digest free mouse DNA in 3 h in identical conditions. DNAs in slots *a* and *b* are from control incubations of nuclei (1 h and 3 h) in the *Bsu* conditions. Slot *g* contains a mouse satellite DNA marker partially digested with *Eco*RII[15]. Analogous patterns were obtained from rat liver nuclei incubated with *Bsu* and micrococcal nuclease.

Bsu in different conditions (W.H. and K. Heininger, unpublished) were absent in both the control experiments. We cannot exclude the participation of endogenous nucleases but this only after a primary action of the restriction nuclease. The quantity of DNA falling into the regular pattern is definitely higher than one would expect on a statistical basis. The material in the 600 nucleotide pair region, for example, constitutes 1–4% of the total digest. If *Bsu* targets were randomly distributed in accessible segments of the nucleosomes of no more than 20 nucleotide pairs length one would expect less than 0.3% of the DNA in the 600 nucleotide pair region. Possible reasons for the higher experimental values could be a non-random distribution of *Bsu* sites or some basic difference between DNA and chromatin in restriction nuclease digestion.

The experiments aiming at the satellite DNA component of the nuclei were based on the finding that mouse satellite DNA is cleaved into a series of characteristic fragments by *Eco*RII[15]. When mouse liver nuclei were digested with *Eco*RII one could indeed detect these fragments at a high enzyme concentration (Fig. 3). This shows that a sizeable proportion of cleavage sites must have been available for the nuclease, otherwise much larger fragments would have been produced instead. It will be interesting to determine in detail how the degree of accessibility of satellite DNA in nuclei compares with that of the other

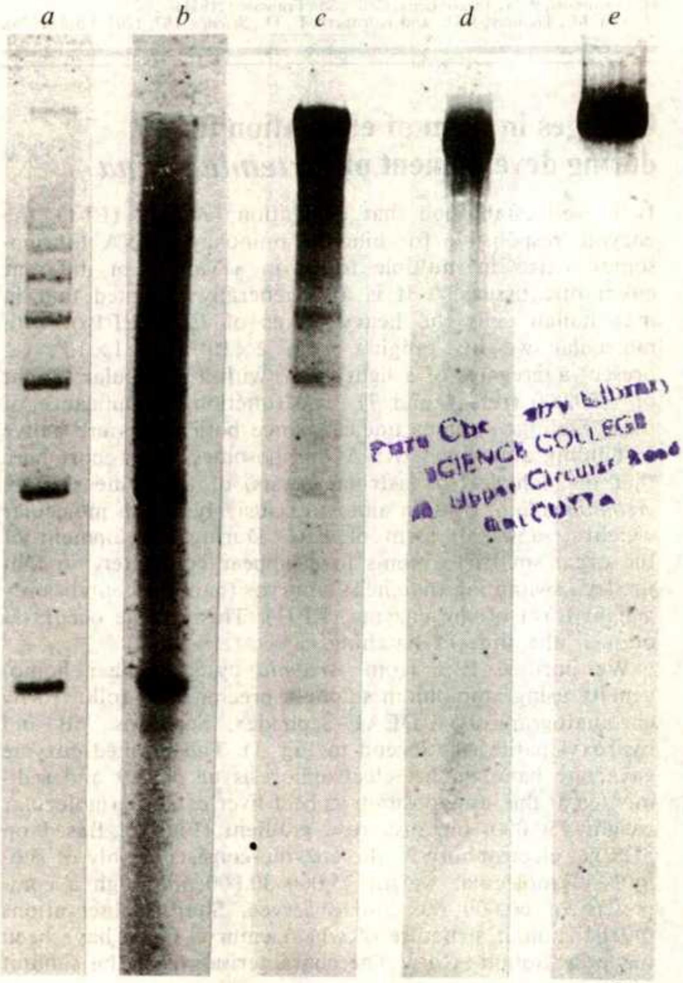

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Fig. 3 Digestion of mouse DNA and nuclei with *Eco*RII. 0.05 A_{260} units of mouse satellite DNA (slot *a*) and 0.2 A_{260} units unfractionated mouse DNA (slot *b*) were incubated with 0.3 × and 1 × *Eco*RII, respectively, for 3 h and the digests analysed on 4% polyacrylamide gels as described previously[16]. *Eco*RII was prepared according to ref. 17. 1 × *Eco*RII is the concentration required to completely digest free DNA in 3 h. In slots *c* and *d*, DNA from mouse nuclei incubated for 3 h with 20 × *Eco*RII and 4 × *Eco*RII in the conditions of Fig. 1 was analysed. Slot *e* contains DNA from control nuclei incubated for 3 h in the absence of nuclease.

DNA and further if the repetitive DNA also conforms to the nucleosome substructure of chromatin.

Our results show that digestion of chromatin with restriction nucleases yields information not available with other nucleases. Note also the possibility of using the more defined digestion of whole nuclei by restriction nucleases for preparative purposes, thus circumventing the random shearing methods used previously. This approach should complement and extend the recently introduced digestion of nuclei with nonspecific nucleases for chromatin preparation[18].

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Changes in form of elongation factor during development of *Artemia salina*

IT is well established that elongation factor 1 (EF1), the enzyme responsible for binding aminoacyl tRNA to ribosomes, exists in multiple forms in a variety of different eukaryotic tissues[1–6]. It is also generally accepted that in mammalian cells the heavy forms of EF1 ($EF1_H$), with molecular weights ranging from 2×10^5 to $>1\times10^6$, represent aggregates of a light form, with a molecular weight of ~50,000 (refs 4 and 7). The functional significance of these findings remains unclear, since both forms are active in binding aminoacyl tRNA to ribosomes. We report here that the dehydrated gastrulae (cysts) of the brine shrimp, *Artemia salina*, contain almost exclusively a high molecular weight (~250,000) form of EF1. During development of the organism $EF1_H$ seems to disappear completely so that the free swimming (hatched) embryos (nauplii) contain only a light form of the enzyme ($EF1_L$). This change occurs at or near the time of hatching.

We purified EF1 from *Artemia* cysts to near homogeneity using ammonium sulphate precipitation followed by chromatography on DEAE–Sephadex, Sepharose 6B and hydroxylapatite (see legend to Fig. 1). The purified enzyme gave one band on gel electrophoresis at *p*H 8.9 and sedimented at the same position as beef liver catalase (molecular weight 250,000) on a sucrose gradient (Fig. 1). Based on SDS gel electrophoresis, the enzyme consists mainly of subunits of molecular weight 25,000–30,000, although a component of 60,000 was also observed. Similar observations on the subunit structure of wheat embryo $EF1_H$ have been made by Bollini *et al.*[8]. The characterisation of the subunit

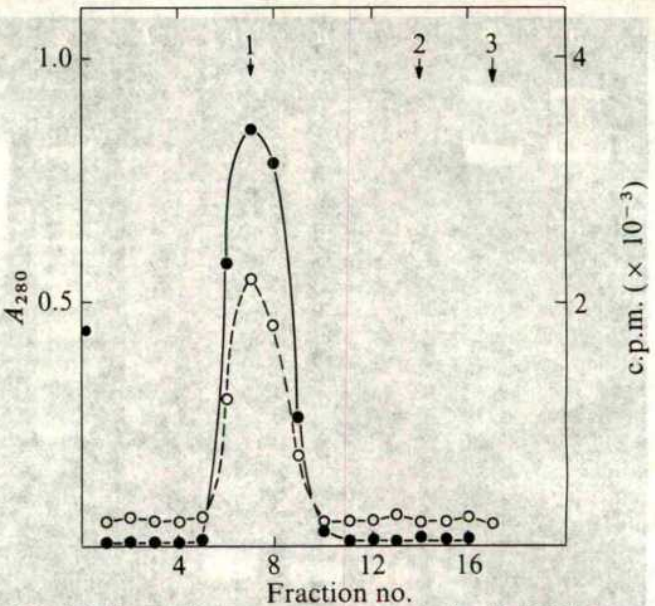


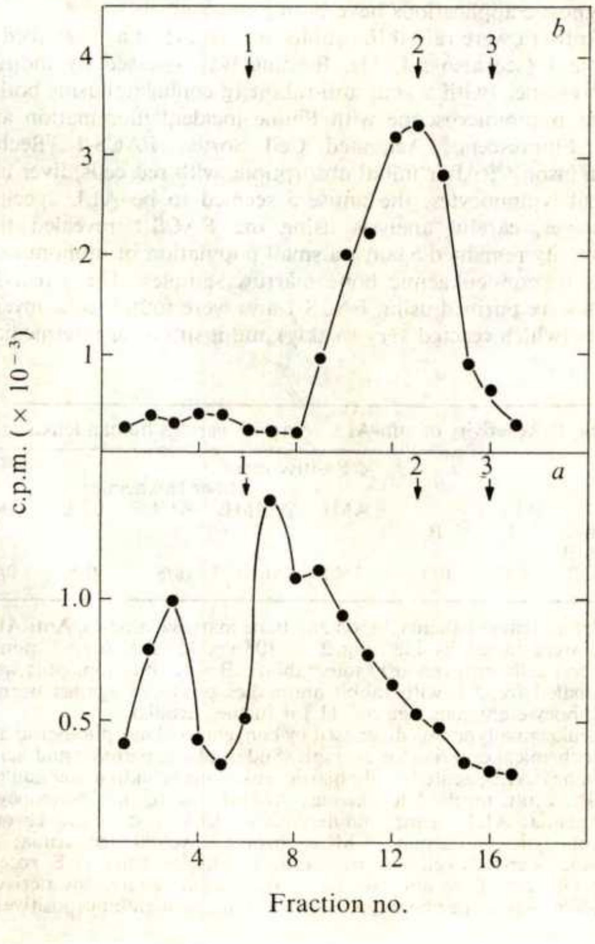
Fig. 1 Sedimentation on a sucrose gradient of purified EF1 from *Artemia* cysts. EF1 was purified as follows: all operations were performed at 2–4 °C. Cysts were washed with NaOCl and water, and ground in a mortar at 0 °C as described[13]. Nauplii were washed extensively with 4% NaCl before preparation of extracts. Both ground cysts and nauplii were suspended in extraction buffer (20 mM Tris-HCl, *p*H 7.5, 100 mM KCl, 10 mM mercaptoethanol, 5 mM magnesium acetate, 5% glycerol (w/v)) and the post-ribosomal supernatant obtained by a series of centrifugations at 2 °C: 5 min at 2000*g*, 10 min at 33,000*g* and finally 90 min at 50,000 r.p.m. (150,000*g*) in the Beckman 60 Ti rotor to pellet the ribosomes. The supernatant from each centrifugation was poured through a glass wool plug. Care was taken to avoid the upper lipid layer after the last centrifugation. The protein fraction of the post-ribosomal supernatant which precipitated between 30 and 70% saturated ammonium sulphate was dialysed against a buffer containing 20 mM Tris-HCl, *p*H 7.5, 150 mM KCl, 10 mM 12-mercaptoethanol, 0.1 mM EDTA and 1 mM magnesium acetate and applied to a column of DEAE–Sephadex G-50 previously equilibrated with the same buffer. EF1 was not retained by DEAE–Sephadex and was collected in the eluate, concentrated by ammonium sulphate precipitation, and chromatographed on Sepharose 6B, using the same buffer as above except that the KCl concentration was 100 mM. The fractions containing EF1 activity were concentrated and, after dialysis against 50 mM KPO_4 buffer, *p*H 7.5, containing 10 mM 2-mercaptoethanol and 10% glycerol, applied to a column of hydroxylapatite (BioRad HTP). The column was developed with a linear gradient between 50 mM KPO_4 and 250 mM potassium phosphate buffer, *p*H 7.5, containing 10 mM 2-mercaptoethanol and 10% glycerol. EF1 eluted at 200 mM phosphate. The active fractions were concentrated by ultrafiltration and rechromatographed on Sephadex G-200. One protein peak was obtained which had a specific activity (one unit of activity is defined as the amount of protein required to bind one 1 pmol of Phe tRNA in the standard assay (see below), ranging from 750-1,000 for different EF1 preparations. Purified EF1 (100 µl of a 1% solution) was applied to 5 ml of a 10–30% linear sucrose gradient in a buffer containing 20 mM Tris-HCl, *p*H 7.5, 100 mM KCl, 10 mM mercaptoethanol and 1 mM magnesium acetate. The sample was centrifuged for 18.5 h at 2 °C and 40,000 r.p.m. in a Beckman/Spinco SW 50.1 rotor. After centrifugation, the tube was pierced at the bottom and 16-drop (~ 0.3 ml) fractions were collected. Aliquots (25 µl) of each fraction were assayed for EF1 activity. Assay mixtures (100 µl) contained the following components: 20 mM Tris-HCl, *p*H 7.5, 100 mM KCl, 10 mM mercaptoethanol, 5 mM $Mg(OAc)_2$, 1.0 A_{280} units of 0.5 M KCl washed 80S ribosomes from *Artemia* cysts[8], 0.25 mM GTP, 20 µg of poly(U) and approximately 35 µg of *Escherichia coli* tRNA charged with 13 pmol (~ 10,000 c.p.m.) of ^{3}H-phenylalanine (1.1 Ci mmol^{-1}). After incubation for 20 min at 30 °C, the reaction mixtures were diluted with ice-cold buffer (20 mM Tris-HCl, *p*H 7.5, 100 mM KCl and 5.0 mM magnesium acetate) and collected by suction on to 0.45-µm nitrocellulose filters (Sartorius). The filters were washed 3 times with 3-ml portions of cold buffer, dried and counted with 30% efficiency in a Packard Tricarb Liquid Scintillation Spectrometer. Marker proteins, designated by the arrows, in a volume of 100 µl were run on a separate gradient and their absorbance measured. 1, Bovine catalase ($S_{20,w}$ = 11.7, molecular weight 247,000); 2, bovine serum albumin (4.3, 66,000); 3, horse heart cytochrome *c* (1.7, 13,400). —, A_{280}; – – – –, c.p.m.

structure of the enzyme is currently under investigation. Our observations regarding the size of the native enzyme in *Artemia* cysts are in agreement with those of Nombela and Ochoa[9].

To establish whether EF1$_L$ is also present in *Artemia* cysts the post-ribosomal supernatant (PRS) of extracts of *Artemia* cysts was analysed for EF1 activity after centrifugation on a sucrose gradient. The results (Fig. 2*a*) show that most of the EF1 activity sediments as high molecular weight forms (S>5), with the bulk sedimenting in a range roughly comparable with that of the purified enzyme.

To determine the form of EF1 which is present in nauplii, *A. salina* embryos were developed for 45 h at 25 °C in 4% artificial seawater. In these conditions >90% of the embryos hatched to form free-swimming nauplii. When the PRS of extracts of nauplii were examined on sucrose gradients, there was a remarkable change in the EF1 pattern (Fig. 2*b*). All EF1$_H$ had disappeared and was replaced by an activity which cosedimented with bovine serum albumin ($S_{20,w}=4.3$), indicating the presence in nauplii of EF1$_L$ exclusively. We next investigated the time during development at which the conversion to EF1$_L$ occurs. *Artemia* cysts were developed as before for 26 h; approximately 40% of the embryos were present as nauplii, as judged by microscopic analysis. Examination of the PRS after 26 h of development on sucrose gradients showed that approximately 40% of the EF1 activity was in the form of EF1$_L$. The remaining activity was present as higher molecular weight forms. Since about 40% of the organisms were free swimming, the distribution of activity could reflect an average value, in which EF1$_L$ exists exclusively in nauplii, whereas EF1$_H$ is present in unhatched cysts. Alternatively, newly emerged nauplii may have the same distribution of the different forms of EF1 as pre-emergent cysts. The exclusive presence of EF1$_L$ after 48 h of growth could then be explained by a gradual conversion of EF1$_H$ to EF1$_L$ during development.

Fig. 2 Sucrose gradient patterns of EF1 activity of cysts and nauplii of *A. salina*. PRS was prepared as described in legend of Fig. 1. Freshly prepared PRS (100 µl) was applied to 5 ml 10–30% sucrose gradients. The gradients were analysed for EF1 activity as described in Fig. 1 using 20-µl aliquots from each fraction (16 drops). In the assay conditions EF1 was limiting. *a*, PRS from cysts (4.4 mg protein per ml) was centrifuged for 16 h at 42,000 r.p.m. in the SW 50.1 rotor; *b*, PRS from nauplii (10 mg protein per ml) was centrifuged for 16 h at 42,000 r.p.m.



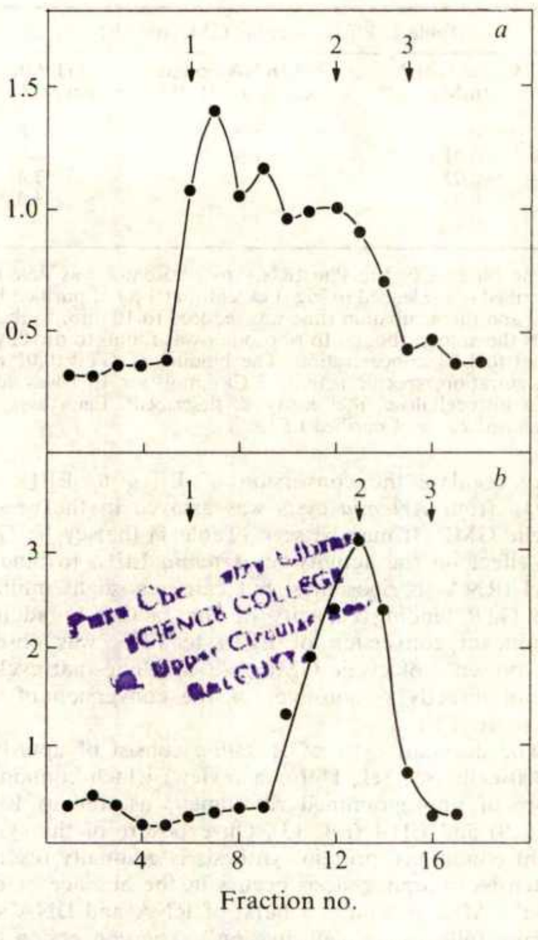


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Fig. 3 Sucrose gradient patterns of EF1 activity after 26 h of development. Extracts were prepared and analysed as described in the legend to Fig. 2. *a*, PRS (100 µl) of *A. salina* after 26 h development, consisting of a mixture of cysts and nauplii (see text), containing 7.2 mg protein per ml was centrifuged at 42,000 r.p.m. for 15 h; *b*, PRS (100 µl) of isolated *A. salina* nauplii after 26 h development (see text), containing 8.2 mg protein per ml was centrifuged at 42,000 r.p.m. for 16 h.

To investigate these two possibilities, cysts were again developed for 26 h, at which time nauplii were separated from developing cysts and pre-emergent nauplii. This separation was accomplished essentially as previously described[14]. The EF1 pattern of nauplii purified thus is shown in Fig. 3*b*; only EF1$_L$ is present in these newly hatched embryos. Consistent with these findings we have observed no significant change in the EF1 patterns during the early development of the cyst (6 h at 25 °C); only after 16 h, when approximately 10% hatching has occurred, is a peak of EF1$_L$ activity clearly visible (unpublished).

We have attempted to convert EF1$_H$ to EF1$_L$ *in vitro*. Purified EF1$_H$ was incubated with varying amounts of PRS from both cysts and nauplii. In preliminary experiments neither supernatant was found to have any effect on the sedimentation behaviour of EF1$_H$. Lanzani *et al.*[10] have reported that cyclic GMP caused a small conversion of EF1$_H$ to a lighter form of the enzyme. In these studies cyclic GMP also seemed to stimulate the binding of GTP by EF1$_H$ as well as factor-dependent polyphenylalanine synthesis. To test the possibility that cyclic nucleotides

Table 1 Effect of cyclic GMP on EF1 activity

Cyclic GMP (mM)	Phe tRNA bound c.p.m. ($\times 10^{-3}$)	GTP bound c.p.m. ($\times 10^{-3}$)
0	1.9	8.6
0.01	1.6	—
0.02	—	7.4
0.04	—	6.3
0.1	1.9	—

The binding of the Phe tRNA to ribosomes was determined as described in the legend to Fig. 1 except that 3 μg of purified $EF1_H$ was used and the incubation time was reduced to 10 min. In these conditions the amount bound to ribosomes was found to directly proportional to EF1 concentration. The binding of GTP (0.01 mM final concentration, specific activity 2 Ci mmol^{-1}) to EF1 was determined by a nitrocellulose filter assay as described[9]. Each assay mixture contained 20 μg of purified $EF1_H$.

may regulate the conversion of $EF1_H$ to $EF1_L$, purified $EF1_H$ from *Artemia* cysts was assayed in the presence of cyclic GMP. It may be seen (Table 1) that cyclic GMP has no effect on the activity of *Artemia* $EF1_H$ to bind aminoacyl tRNA to ribosomes, but causes a slight inhibition of the GTP binding capacity of the factor. In addition, no significant conversion of $EF1_H$ to $EF1_L$ was observed in the presence of cyclic GMP. We conclude that cyclic GMP is not directly responsible for the conversion of *Artemia* $EF1_H$ to $EF1_L$.

The dormant cysts of *A. salina* consist of approximately 4,000 cells (see ref. 11 for a review) which contain a large store of unprogrammed ribosomes[12] as well as EF1, EF2 (ref. 9) and E1F1 (ref. 13). On exposure of the cyst to the right conditions, protein synthesis is gradually resumed and extensive morphogenesis occurs in the absence of cell division[11]. After hatching, a burst of RNA and DNA synthesis occurs followed by cell division[14]. The conversion of $EF1_H$ to $EF1_L$ seems to coincide with these later events. Although we have not shown a direct precursor–product relationship between $EF1_H$ and the $EF1_L$ of nauplii, it seems likely that such a relationship, in which $EF1_H$ represents a storage form of the enzyme, exists. The mechanism by which $EF1_H$ is converted to $EF1_L$ is at present under study.

The coincidence between the conversion of $EF1_H$ to $EF1_L$ and the rapid synthesis of nucleic acid also raises the possibility that EF1, as is the case with its bacterial counterpart TuTs[15], has a function in metabolism other than in protein biosynthesis. Certainly the observation, that the form of EF1 present in all cells of a multicellular organism is correlated with a morphogenetic event, suggests that the occurrence of these forms has real physiological significance. This work was supported by a grant from the US National Science Foundation.

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Candidate leukaemia-specific antigen in man

IT is well established that distinct changes in the antigenic phenotype of the cell surface accompany viral transformation. The 'new' determinants may be viral, oncofoetal (or 'phase' specific) or tumour specific[1–3]. The identification of neoantigens, and particularly putative tumour specific antigens, on human neoplasms opens up possibilities of early and precise diagnosis, patient monitoring and immunotherapy. It may also eventually provide information relevant to the aetiology of the disease. We report here the demonstration of a cell surface antigen present on leukaemic cells from patients with acute lymphoblastic leukaemia (ALL).

Studies with cell surface differentiation markers for distinctive lymphocyte populations have been useful in analysing the phenotype and possible origin of human leukaemic cells (reviewed in refs 4 and 5). ALL seems to be a heterogeneous disease which may, nevertheless, be divisible into two major categories. Approximately 25% of ALL cases have a distinctive T-cell surface phenotype often associated with a mediastinal (thymic) mass or enlargement. The remainder and majority (75%) of the cases seem to lack reactivity with all available cell-surface markers for T and B lymphocytes. Relatively rare cases (1–2%) have a B-lymphocyte phenotype and are considered to be a 'Burkitt lymphoma type'[6] on morphological grounds. It is therefore possible that most patients with ALL have a neoplastic disease which originates in a relatively undifferentiated cell which is either committed to the lymphoid developmental pathway or is a stem cell with wider potentialities.

Several laboratories have reported encouraging results with heteroantisera to leukaemia-associated antigens[7–11]. We have raised antisera to leukaemic blasts from the major class of ALL (that is 'non-T, non-B') and have used these sera to define leukaemia-associated or leukaemia-specific cell surface determinants. Detailed characteristics of these sera and their diagnostic applications have been published[5,11,12].

Antisera were raised in rabbits and absorbed as described in Table 1 (see also ref. 11). Binding was assessed by indirect fluorescence (with a goat anti-rabbit Ig conjugate) using both a Zeiss photomicroscope with Plume incident illumination and the Fluorescence Activated Cell Sorter, FACS-1 (Becton Dickinson)[13]. After initial absorptions with red cells, liver and tonsil lymphocytes, the antisera seemed to be ALL specific; however, careful analysis using the FACS-1 revealed that reactivity remained against a small population of mononuclear cells in non-leukaemic bone marrow samples. These reactive cells were purified using FACS-1 and were found to be myelocytes (which reacted very weakly) and a subset of intermediate

Table 1 Reactivity of anti-ALL sera with various human leukaemias

% Positive cases							
ALL			Other leukaemias				
Non-T Non-B	T	B	AML	AMML	AUL	CLL	CML
47/50	0/13	0/3	1/48	0/10	6/8	0/6	0/5

All untreated patients, blood and bone marrow samples. Anti-ALL sera were raised by injecting 2×10^8 viable ALL (non-T non-B variety) cells intravenously into rabbits. Before injection, cells were incubated for 2 h with rabbit antibodies produced against normal lymphocyte antigens (see ref. 11 for further details).

Leukaemia type was diagnosed by conventional morphological and histochemical criteria (for example, Sudan black, periodic and Schiff staining). ALL, acute lymphoblastic leukaemia (children and adults); AML, acute myeloid leukaemia; AMML, acute myelo-monocytic leukaemia; AUL, acute undifferentiated leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; T, (thymus derived) cell-like membrane phenotype (that is E rosette positive, anti-T serum positive[19]; B, ('bursa' equivalent derived) cell-like membrane phenotype (that is anti-immunoglobulin positive)[19].

Table 2 Possible antigenic specificities defined by antisera to ALL

Potential antigen	Cells tested	Cross reactive with anti-ALL*
'Normal' antigen	Thymus†, tonsil†, appendix, blood WBC, spleen, bone marrow, liver	No‡
'Cryptic' normal lymphocyte antigen	Tonsil lymphocytes treated with trypsin§, Pronase or neuraminidase¶	No
Lymphocyte antigens expressed during mitosis	Mitogen activated tonsil T and B lymphocytes**	No
	Lymphoblastoid cell lines††	
	Lymphoblasts from blood of patients with infectious mononucleosis	•
'Foetal' antigens	Spleen 13–22 weeks Thymus 15–22 weeks Liver (9–22 weeks)†	No

* All cells were tested by indirect immunofluorescence with anti-ALL.

† Cells also tested for their capacity to absorb out anti-ALL antibodies.

‡ No reactivity = less than 0.5% positive cells recorded which is the 'background' with normal control sera, or alternatively, in absorption experiments, reflects no shift in the FACS profile on anti-ALL against ALL cells.

§ Trypsin (Sigma 12,000 BAEE U mg^{-1}, twice crystallised) 50 μg per 10^6 cells per ml per 30 min per 37 °C.

¶ Pronase (BDH 45,000 PuK units per g) 50 μg per 10^6 cells per ml per 30 min per 37 °C.

¶ Neuraminidase (*Vibrio cholera*, Calbiochem, grade B) 0.5 U per 10^6 cells per ml per 30 min per 37 °C.

** Tonsil T lymphocytes cultured with phytohaemagglutinin or concanavalin A or spleen B lymphocytes cultured with pokeweed mitogen (see ref. 20 for method).

†† Derived from normal adult blood or from blood of patients with infectious mononucleosis, Burkitt's lymphoma or ALL (see ref. 11)

normoblasts (which reacted very strongly)[12]. Most intermediate normoblasts in early (11–13 week) foetal liver were also strongly stained. The former cross reactivity could be removed by absorption with AML or bone marrow and both activities could be removed with either bone marrow or foetal liver. After this activity had been removed, staining of ALL cells remained and could not be significantly diminished by further absorption[12].

The reactivity of bone-marrow-absorbed anti-ALL sera with various leukaemias is given in Table 1. Virtually all non-T, non-B ALL cells react, whereas no T-cell ALLs or myeloid leukaemias stain. It is particularly interesting that six of eight cases of acute undifferentiated leukaemias (AUL) were positive (Table 1) as well as four of twelve cases of chronic granulocytic leukaemia (CGL) in blast crisis relapse (unpublished results of T. Revesz, G. Janossy, M.F.G., and M. Beard). These results suggest that these leukaemias share a common antigen which might reflect their origin from a common progenitor or leukaemia 'target' cell. ALL patients in remission have been studied and in a minority some staining cells were detectable[12]. The possible significance of this result with respect to monitoring patients throughout treatment and predicting relapse has been discussed elsewhere[5,12].

The results suggested the existence of a leukaemia-specific antigen. We have therefore sought to eliminate unequivocally alternative interpretations. These are that the antigen is (1) foetal or oncofoetal; (2) a cell-cycle restricted determinant; (3) a normal cryptic determinant, or (4) a normal antigen present on a rare cell, for example, a stem cell. The first three of these were relatively straightforward to assess; the cell types tested are listed in Table 2. Within the sensitivity of our absorption and immunofluorescence tests, no evidence could be found to suggest or support a foetal, cryptic or cell-cycle association of the ALL antigen (Table 2). The fourth possibility is difficult to test. We can conclude that if the antigen is present on 'normal' cells in marrow then the frequency of reactive cells is certainly less than 0.5% and probably less than 0.1%. This level of detection is probably insufficient to exclude stem cells. This question has not been considered in previous analyses of

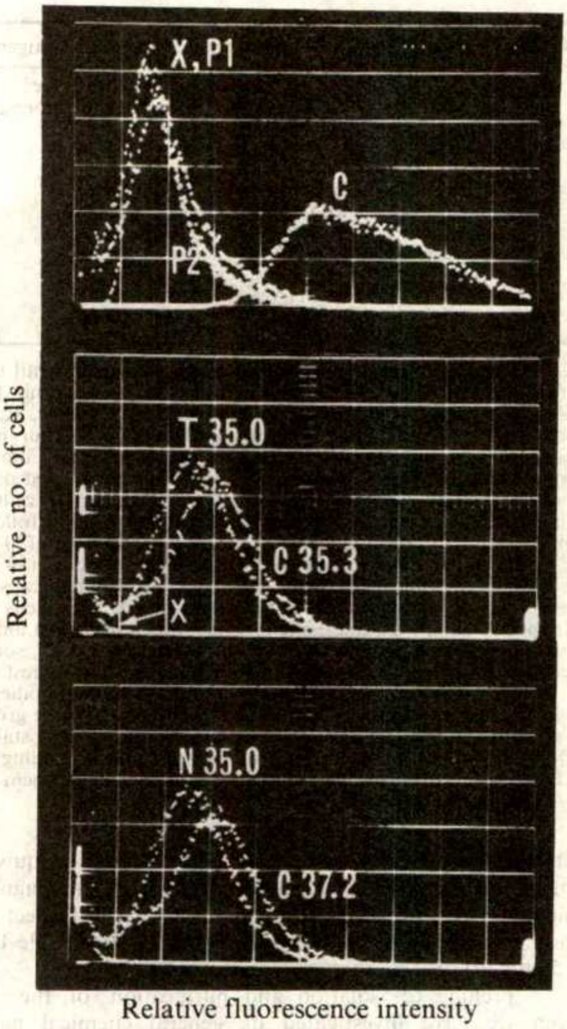

Fig. 1 Effect of Pronase, trypsin and neuraminidase on ALL associated antigens. FACS fluorescence profiles. A stock solution of Pronase (40 mg ml^{-1} BDH 45,000 PuK units per g) was prepared in PBS. Two Pronase concentrations were used—1 mg per 10^6 cells per ml and 10 mg per 10^6 cells per ml. The cells were incubated with the enzyme for 1 h at 37 °C after which the enzyme was inactivated by the addition of 50 μl of foetal calf serum and incubating the cells at room temperature for 10 min. The cells were washed once with Earle's saline containing 10% foetal calf serum and tested for positivity with an anti-ALL serum, using the indirect immunofluorescence assay. FACS fluorescence profiles were compared for Pronase-treated and untreated cells or cells to which Pronase had been added and immediately inactivated. The viability of cell suspensions after treatment with Pronase, as assessed with Trypan blue, was > 99%. Similar experiments were carried out with trypsin. A 0.25% stock solution of trypsin (Sigma, 12,000 BAEC U mg^{-1}) was prepared in PBS. Time course and dose–response experiments were carried out using trypsin concentrations from 0.12 mg per 10^6 cells per ml to 1.2 mg per 10^6 cells per ml and incubation times from 0.5 to 1.5 h. The trypsin was inactivated by adding 50 μl of foetal calf serum and incubating the cells at room temperature for 10 min. The viability of all cell suspensions after trypsination and as assessed with Trypan blue was > 99%. A stock solution of neuraminidase (500 U ml^{-1}, *Vibrio cholera*, Calbiochem grade B) was prepared in PBS. Neuraminidase was used at concentrations of 0.5, 2.5, 5.0 and 10.0 U per 10^6 cells per ml. Cells were resuspended in calcium saline and incubated with neuraminidase for 20 min at 37 °C. The viability of cell suspensions after treatment with neuraminidase was > 99%. C, ALL cells tested with anti-ALL serum; X, ALL cells tested with normal rabbit serum; P, ALL cells after Pronase treatment (P1, 1 mg per 10^6 cells per ml; P2.10 mg per 10^6 cells per ml) and tested with anti-ALL serum. The fluorescence profiles in the above cases were moved to the right of the figure by increasing the laser power. This clearly demonstrates the similarity between P1, P2 and X. The following were recorded at the routine laser power: T, ALL cells after trypsination (1 h, 1.2 mg per 10^6 cells per ml) and tested with anti-ALL serum; N, ALL cells after neuraminidase treatment 20 min, 10 U per 10^6 cells per ml) and tested with anti-ALL serum. The numbers on the figures indicate the number of positive cells per 40,000 cells analysed.

Table 3 Co-capping of lectin binding sites and ALL antigen

	Assay for uncapped sites	
	Ligand	% Cells with ring fluorescence
Lentil sites capped (64.8%)	Lentil	< 1
	Anti-ALL	< 1
Control	Lentil	100
	Anti-ALL	98
PHA sites capped (70.4%)	PHA	< 1
	Anti-ALL	> 99
Control	PHA	100
	Anti-ALL	98

Lens culinaris haemagglutinin (lentil) was purified from lentil using the method of Howard *et al.*[21]. Purified phytohaemagglutinin from *Phaseolus vulgaris* was obtained from Burroughs Wellcome. Rabbit antisera to PHA and lentil were prepared by two to four intramuscular or subcutaneous injections of 1 mg of lectin in Complete Freund's adjuvant. The co-capping experiments were carried out as follows. ALL blasts were treated with PHA (10 μg per 10^6 cells per ml) or lentil (0.5 mg per 10^6 cells per ml) for 30 min at 37 °C followed by appropriate saturation dilutions of rabbit anti-lectin sera (30 min, 37 °C) then fluorescein-labelled goat anti-rabbit antibody (30 min, 37 °C). Redistribution of lectin binding sites into discrete caps was observed and the number of capped cells was noted. These cells were then divided into two samples, one of which was tested in an indirect immunofluorescence assay at 4 °C in the presence of 0.2% sodium azide for reactivity with anti-ALL serum. The second was restained with lectin, rabbit anti-lectin and goat anti-rabbit antibodies, at 4 °C in the presence of 0.2% sodium azide. In both of these groups, cells pre-capped with lectin were assessed for additional ring staining which would indicate the presence of uncapped lectin binding sites or ALL antigen. Controls were untreated before reacting them with anti-ALL and lectin.

putative leukaemia-specific reagents. The lack of unequivocal proof of leukaemia specificity does not preclude the diagnostic application of the antiserum, and the results in this respect have already proved of considerable interest and value (Table 1 and refs 5 and 12).

As a prelude to isolation and purification of the ALL antigen we have investigated its general chemical nature. Treating ALL cells with enzymes (Fig. 1) revealed that the antigen was associated with a Pronase-sensitive but trypsin- and neuraminidase-insensitive structure.

To test whether the antigen was associated with a oligosaccharide-bearing molecule we investigated the capacity of lectins to redistribute the antigen within the lateral plane of the membrane. This 'co-capping' approach has proved valuable in revealing molecular interrelationships of cell-surface molecules[14–16]. The design of these experiments and the results observed are given in Table 3. It seems that lentil (*Lens culinaris*) lectin interacted (and co-capped) with the molecular entity on which the ALL antigen was expressed. In contrast, phytohaemagglutinin (*Phaseolus vulgaris*) binding sites and the ALL antigen seemed to be independent. We therefore suppose that the molecule of interest is probably a glycoprotein, although a strong non-covalent association with glycoprotein cannot be excluded.

The possibility that a virus is involved in human leukaemia is clearly of great potential importance. The most suggestive evidence so far comes from the isolation by Gallagher and Gallo of C-type particles from long term tissue culture of myeloid cells from an AML patient (HL23)[17]. A viral isolate from this patient, called HL23V–1, has been shown to be infectious for various cell types and to resemble closely the C-type simian sarcoma virus (SSV) isolated from a woolly monkey[18].

We have therefore attempted to determine immunological cross reactivity between the ALL antigen described above, HL23V–1 and a number of other candidate human and animal leukaemia viruses. These experiments involved asking two related questions. First, can virus extracts absorb the anti-ALL activity? Second, do antisera to these same viruses react with ALL cells? The latter experiment is controlled by demonstrating reactivity with infected animal cells. These tests were carried out under quantitative conditions using FACS analysis. The results showed that there is no immunological cross reactivity demonstrable by absorption between the ALL antigen and murine leukaemia virus (Gross and Moloney), feline leukaemia virus, HL23V–1, Newcastle disease virus and influenza virus (X31). Antisera to disrupted feline leukaemia viris (GaFeLV) which reacts with interspecies determinants on the P30 molecule and antisera to simian sarcoma virus P30 (RaSSVP30) or whole SSV showed no fluorescent labelling of ALL cells. Neither did antisera to Epstein–Barr virus, herpes saimiri, rubella and cytomegalovirus.

In spite of this impressive list of negatives we cannot and do not exclude viral involvement in ALL or in the expression of the ALL antigen. Clearly, however, these results diminish the possibility that the ALL cell-surface antigen we have identified is a virally coded determinant.

This research was supported by the Imperial Cancer Research Fund (M.G., N.H.) and the Medical Research Council (G.B.). We thank many colleagues for viruses and anti-viral sera, especially Drs N. Teich and R. Weiss (HL23V–1), Dr. O. Jarrett (FeLV), Dr J. Skehel (X31), Dr A. Rickinson (EBV and herpes saimiri), Dr W. Marshall (CMV, rubella). Dr Teich also carried out absorptions of anti-ALL sera with HL23V–1. Drs A. Lister, D. Catovsky, and N. Rapson supplied human leukaemic cells.

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Corrigendum

In the article "Differential effects of glucocorticoids on DNA synthesis in normal and virus transformed chick embryo cells" by D. W. Fodge and H. Rubin (*Nature*, **257**, 804; 1975) the right-hand axis of Fig. 3 is incorrectly labelled. It should read Labelled nuclei (fraction) and not (%) as printed.

Erratum

In the Matters Arising contribution "Sympathoadrenal medullary activity in young, spontaneously hypertensive rats" by H. Grobecker, M. F. Roizen, V. Weise, J. M. Saavedra and I. J. Kopin (*Nature*, **258**, 267; 1975) the asterisked footnote to Table 2 should read ... *Results are expressed as μg of catecholamines, μg of noradrenaline and units of enzyme per adrenal gland and are the mean values (±s.e.m.) for groups of 14 animals ... and not as printed.

matters arising

On the 27-day cycle in the rainfall at Los Angeles

ROSENBERG and Coleman[1] reported a 27-d cycle in the rainfall of Los Angeles. Subsequently the former author has used the cycle to forecast, starting from an initial day to be determined each year, 13.5 d wet and 13.5 d dry periods in the winter months of Southern California. Their 27-d cycle was based on the spectral analysis of the rainfall data at the Los Angeles Civic Center. They used the Blackman–Tukey method with the Tukey spectral window[2] and a maximum of 250 lags. I question the significance of the peaks in the Los Angeles rainfall spectrum.

Professor M. Wurtele of the Department of Meteorology at UCLA provided a record of daily rainfall data for Los Angeles covering a period of over thirty years (June 1943–August 1973). (UCLA campus is ≈ 17 miles from the Los Angeles Civic Center.)

Spectral analysis of the UCLA rainfall data was done by the method of direct estimation of spectral density using Fast Fourier Transforms[3]. The detailed description of this method is found in Cooley *et al.*[4].

Figure 1 shows the normalised density spectrum of the UCLA data. It has the general red noise (spectrum) feature (that is, most of the energy is at low frequencies) which is typical of geophysical phenomena.

This spectrum shows peaks around 28.4, 22.3 and 16.0 d. The first peak whose period is comparable to the 27 d of Rosenberg and Coleman is the most pronounced. The question, however, is whether or not this peak is statistically significant.

It is generally assumed that each of the spectral estimates has a χ^2 distribution with an equivalent number of degrees of freedom (e.d.f.). The e.d.f. is a function of the total length of the record N and the maximum number of lags (or, equivalently, of half the width of the window of the power spectrum).

Having determined the e.d.f., the significance of any of the peaks depends on whether or not persistence in the data is taken into account. This is done by comparing the density of the peak multiplied by the e.d.f. to the density of the red noise (spectrum) at that particular frequency, rather than to the density of the Gaussian white spectrum[5].

Red noise (spectrum) is the spectrum of the persistence-modelling random process referred to as the first order Markovian (or autoregressive) stationary process.

On the basis of our analysis all three peaks were not significant relative to the red noise (spectrum) even at the 80% confidence level which Rosenberg and Coleman arbitrarily chose. The confidence level for the 28.4-d peak is 1%. The confidence levels for the 22.3-d peak and the 16-d peak are 15% and 75% respectively.

We are therefore led to believe that the spectral peak around 28.4 d is a spurious one. Its statistical significance hinges on whether or not the reference spectrum, with which it is compared, takes into account persistence in the rainfall data.

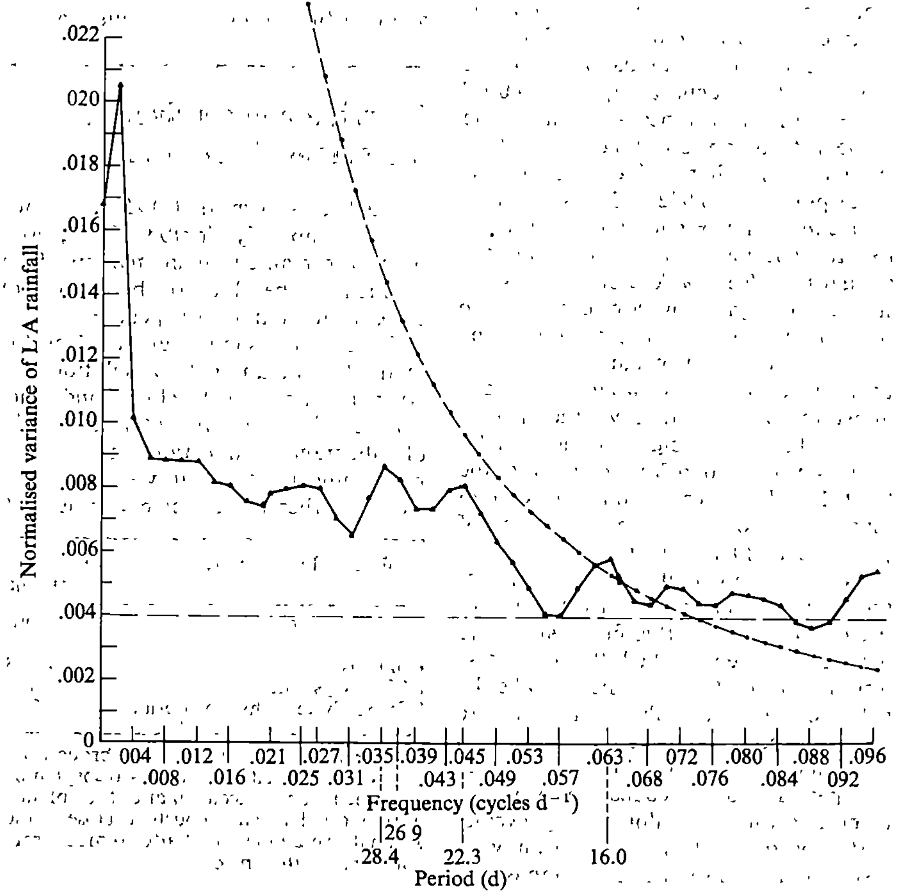

Fig. 1, The normalised variance of Los Angeles rainfall (the area under the curve is unity). The data window used is

$$W(J) = 1 - \left(\frac{J-(L-1)/2}{(L+1)/2}\right)^2; j = 0,1,\ldots,L-1$$

which gives a sharp spectral window very close to the shape of the spectral window of a cosine arch[4], and results in an e.d.f $\approx 3\cdot25N/L = 83$ (ref. 3), where L is the length of the data segment for which the sample spectra are computed. The horizontal line (— — —) represents the white spectrum, while the curve (—.—.—.—.) is for a red spectrum with decay coefficient (ρ) = 0.89.

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ROSENBERG REPLIES—El-Sayed has analysed a portion of the daily rainfall spectrum shown in his Fig. 1 by comparison with a 'red noise' spectrum. In the frequency range of interest, however, the plotted red noise Markovian curve does not fit the data. He has stated (private communication) that he used a least squares fit to the pre-whitened rainfall spectrum over frequencies up to 0.5 c d^{-1}. Such a fit (which determines ρ) is overwhelmingly weighted by the high frequency power from 0.1 to 0.5 c d^{-1}, not shown in Fig. 1. To be valid, the red noise reference spectrum should fit the data equally well in the frequency interval whose peaks are being questioned and in the rest of the spectrum. For example, in the article by Gilman *et al.*[2] the Markovian curve used to approximate rainfall data fits all frequency intervals equally well.

El-Sayed (private communication) claims that the pre-whitening he used eliminated the annual peak. Pre-whitening (flattening the average slope of the spectrum) and recolouring should result in a spectrum with peaks (including the annual peak) whose height above the continuum is about the same as in the un-pre-whitened spectrum, but with a reduction of power leakage into the continuum. No sharp band pass filter was used to eliminate the annual peak because it is clearly evident at 1/365 = 0.0027 c d^{-1} in Fig. 1. The peak-like shape and the very steep slope on its right side, which levels out immediately, indicates that the value plotted at 0.002 c d^{-1} is not part of a continuum. Figure 1 (ref. 1), leads to the spurious conclusion that the annual peak (see Table 2 of ref. 3) is not statistically significant with respect to the red noise test. If some process applied by El-Sayed reduced the height of the annual peak, which otherwise would have been above the Markovian curve, then it may have reduced the height of the ≈ 27-d peak as well. A good Markovian fit to the 0.0–0.1 c d^{-1} interval could have been made by choosing the same number of points to be fit in this interval as in the 0.1–0.5 c d^{-1} interval. The one point at the known annual periodicity could have been excluded from this fit. In short, we believe El-Sayed applied the red noise test incorrectly. He also failed to take the cube root of the daily rainfall (before doing a spectrum) which is considered absolutely necessary to obtain a meaningful spectrum[4] as it reduces the variance associated with anomalously large events.

If a spectral peak is not significant with respect to red noise, the conclusion is that this peak should "be relegated to the category of probable accidents that might not recur in an independent data sample"[2]. Yet the 27-d peak occurs in each of the 5 spectra shown in Figure 2 of ref. 3. These spectra include the totally time-independent spectra for Los Angeles for 1900–35 and for 1936–71, each with 105 degrees of freedom, and spectra (each with 129 degrees of freedom) for 1927–71 for the cities of Los Angeles, Santa Barbara, and San Diego. The latter two cities are 300 km apart. The occurrence of the 27-d spectral peak in long, time-independent intervals is one of the most significant tests of its reality, and supports our criticism of El-Sayed's analysis.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

In our study we searched for a possible correlation between rainfall and the new moon or full moon cycle (result negative) but no other lunar cycle was examined. Using data covering 20 yr, Bryson[5] found a statistically significant correlation between the latitude of the surface, semi-permanent, East Pacific high pressure cell and the meridional lunar force over the 27.3-d lunar cycle in declination. During this cycle, the moon moves north in declination for 13.65 d and then south for 13.65 d and, as Bryson found, pulls the Pacific high cell along with it. Lamb[6] examined this result and stated that "the association appeared to be statistically significant". This high pressure cell acts in turn as a classical blocking high, blocking storms from reaching Southern California as in Fig. 1 of our study[3].

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Cysteine and survival of transplanted thymoma

It has been shown[1] that 0.25 M cysteine could completely inhibit the growth of a transplanted thymoma, an effect claimed to be due to the ability of cysteine to inhibit collagenase.

Cysteine is usually purchased as the hydrochloride and 0.25-M solutions are strongly acidic (pH~1.0), and, not surprisingly, cause necrosis at the site of subcutaneous injection. In the paper by Campbell *et al.*[1] it was not made clear whether the cysteine was neutralised before injection and since isotonic saline was used as a control solvent, it is probable that the cysteine was not, in fact, neutralised.

Table 1 shows that a solution of cysteine hydrochloride can readily prevent the growth of a transplanted plasma cell tumour, whereas neutralised cysteine has only a very slight inhibitory action. In the experiment described in Table 1 the antitumour effect observed is thus due, almost entirely, to the injection of an acidic solution rather than to any special property of cysteine.

Table 1 Effect of cysteine and cysteine hydrochloride on the growth of the transplanted PC6 plasma cell tumour

Treatment	Tumour weight (g)	Inhibition (%)
Controls	9.8, 8.1, 8.4, 9.0, 8.6	
0.25 M Cysteine*	5.6, 5.5, 5.6, 7.7, 5.3	33
0.25 M Cysteine hydrochloride	0.3, 1.6, 0, 0.6, 0	94

*The solution of cysteine was brought to pH 7.5 by titration with normal NaOH.

The plasma cell tumour was transplanted subcutaneously as a tumour fragment by trocar. Treatment commenced 3 d after transplantation. Samples (0.2 ml) of the appropriate solutions were injected subcutaneously as close to the tumour site as possible for five consecutive days. Animals were killed 35 d after tumour transplantation and the weight of the treated tumours compared with controls.

It should also be noted that cysteine hydrochloride given intraperitoneally to mice as an unneutralised solution is between five and ten times more lethal than neutralised cysteine.

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CAMPBELL ET AL. REPLY—Whereas we did not attempt to buffer our cysteine solutions and acknowledge the possibility raised by Connors and Jones[1], the peak of activity that we consistently obtained at 0.25 M cysteine suggests that a specific effect is also involved.

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reviews

Lots of potential

Electric Current Flow in Excitable Cells. By J. B. Jack, D. Noble and R. W. Tsien Pp. xvi+502 (Clarendon: Oxford, Oxford University: London, August 1975.) £18.00.

EXCITABLE tissue, that is, nerve and muscle, should not be tampered with lightly. True, there are all sorts of reasonable humanitarian incentives such as the existence of schizophrenia or heart failure but there is the lurking danger that the cells will take over and become the passion and obsession of the investigator. Generally a physiologist and often inadequately trained in physics and mathematics, he seems from then onwards doomed to a life of frequently frustrating self-education in those disciplines. He should find this book very helpful.

The first half is devoted to applications of linear cable theory. It is an unfortunate fact that even the simplest model of a nerve fibre (which does not even attempt to stimulate the biologically important process of lossless transmission) leads to a partial differential equation needing operational or transform methods for its solution. To follow this part of the book the reader must reconcile himself to the need to be familiar with the Laplace transform method. This done, however, he will find a very clear account of the classical study of nerve by Hodgkin and Rushton, originally published in 1946, its application to the analysis of endplate potentials by Fatt and Katz and its elaboration by Rall to synaptic potentials in central neurones regarded as geometrically non-uniform cables The authors' intention was evidently not to produce a neat compendium of formulae for immediate numerical exploitation On the contrary, there is a certain deliberate and, I think, attractive untidiness about the treatment, some results being stated without proof, others being derived in several different ways with different degrees of completeness, some points are dwelt on because of their mathematical, and others because of their physiological interest.

The second half of the book is concerned with action potentials. Dealt with in turn are the non-linear electrical properties of membranes—which are, of course, what make action potentials possible—the excitation process, conduction and repetitive activity. As is well known, non-linear differential equations do not in general succumb to analytical methods, but as the authors show in their last chapter, such methods can produce significant information if the function representing the electrical behaviour of the membrane is chosen with sufficient ingenuity. On a first reading, this half of the book was somewhat less satisfactory. Parts of the chapter on non-linear membrane properties seem unnecessarily difficult to follow and the reader is too frequently directed to external references for points of substance. In the chapter on repetitive activity, the theoretical discussion sometimes seems too weighty for the experimental data. These are minor criticisms, however, in the light of the enormous amount of material presented.

The book is extremely well written at a detailed level. It is also well produced: among the very few mathematical misprints I noticed, only one, the omission of a pair of brackets from equation 3.60, was catastrophic. In their introduction the authors write that they were primarily motivated “ . . . by the desire to make what is admittedly one of the most difficult areas of physiological theory less forbidding and more accessible . . . ”. I think that they have succeeded.

B. L. Ginsborg

Immunological use for hormones

Immunisation with Hormones in Reproduction Research. (Proceedings of the International Symposium.) Edited by E. Nieschlag. Pp. x+253. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl.55; $22.95.

THIS book presents to a wider audience the papers prepared for a small international symposium held in March of this year. There are three introductory papers on the immune response, autoimmune phenomena and side effects of immunisation by insulin as a model system. All will be of interest to endocrinologists whose knowledge of immunology requires updating. Eight papers are grouped under the heading “Basic Research”, and vary considerably in scope, content and value, ranging from quite lengthy reviews of original work and the published literature through excellent short reports of well-defined, although limited, projects to scrappy accounts of rather diffuse studies, so incomplete in performance or reporting (or both) as to be of little value. The four concluding papers deal with attempted or projected practical applications and here the high standard set by the introductory papers is maintained. Topics discussed are: immunisation with a steroid pheromone–protein conjugate to control the development of boar taint in maturing intact male pigs; immunisation with gonadotrophins to control reproduction in dogs; the prospects for and early experiments on the control of human fertility by immunisation with the protein hormone produced by the early human cenceptus chorionic gonadotrophin; and a brief account of the use of homologous antibodies raised in a primate against pituitary luteinising hormone from a subprimate to reversibly control the development of the corpus luteum in sterile cycles and to terminate pregnancy in fertile cycles in other members of the same primate species.

The papers were clearly written by enthusiasts yet suprisingly the common feature of many of them is the emphasis on the difficulties and uncertainties of both the practical procedures and of the interpretation of the results obtained. This impression might well have been stronger had the discussions after each paper been reported in full rather than in the form of a quite unrewarding and frustrating synopsis. Nonetheless this is a useful book for any biologist who wants a recent account of work in an area which is still of great interest even though it seems to be rather less promising, in terms of either basic research or practical application, than it was hoped it would be. The production of the book, from the remarkably error-free typescripts supplied by the participants, is excellent and has been commendably prompt

K. Brown-Grant

Gas-phase microwave spectroscopy

Molecular Rotation Spectra. By H. W. Kroto. Pp. xi+311. (Wiley-Interscience: London and New York, March 1975.)

THERE are at least seven books on gas-phase microwave spectroscopy. The subject provides many very satisfying examples of successful application of quantum mechanics to models which are simple (for example the rigid rotor) yet fit experimental microwave data in a convincing manner. Furthermore, these theoretical treatments have made possible the determination of reliable structures, dipole moments, field gradients, barriers to internal rotation, molecular quadrupole moments, force constants, conformations, and so on, for a large number of molecules. The classic book, in this reviewer's opinion, remains that of Townes and Schawlow *(Microwave Spectroscopy)* which is, however, now twenty years old. A more recent work of extensive coverage is that of Gordy and Cook *(Technique of Organic Chemistry,* 1972). Any additional book has to compete with a number of very excellent volumes.

Kroto covers most of the usual areas: energy levels of the rigid rotor, selection rules and intensities, Stark effect, nuclear quadrupole interactions, internal rotation, vibration–rotation interaction, linear molecules with electronic angular momentum, and, rather briefly, experimental methods.

There are reports of recent work not contained in earlier books—notably the contributions of Watson to the molecular Hamiltonian and Longuet-Higgins to the symmetry classification. Further, the general approach is somewhat different. Instead of complete coverage of the many possible topics with references to the literature for derivations, Kroto has omitted a number of effects (for example, the Zeeman effect of diamagnetic molecules) but has attempted to present more complete derivations. It is a highly personal judgement as to whether he has succeeded in clarifying all these derivations; certainly for many, more elementary treatments are available On the other hand, the book provides excellent examples of more advanced methods The first chapter is mainly devoted to quantum mechanical principles but it seems doubtful that it would be too helpful to someone who had not already mastered the basic ideas.

This book has more than the usual space devoted to some of the practical methods of analysing spectra and has many figures of well chosen spectra to illustrate various points.

Altogether, this new work is a welcome addition to the field but is not a substitute for the larger books with more complete coverage, more tables, larger bibliographies, and so on.

E. Bright Wilson

Catalysis, great and small

Catalysis in Micellar and Macromolecular Systems. By Janos H. Fendler and Eleanor J. Fendler. Pp. xii+545. (Academic: New York and London, July 1975.) $44.00; £21.10.

SINCE the pioneering work of Duynstee and Grunwald was published in 1954 there has been a growing interest in the catalysis of organic reactions by micelles. Much of this interest has been generated by the hope that micelles might mimic enzymes in their catalytic powers. Unfortunately, although the Michaelis–Menten mechanism is useful for the description of the kinetics of micelle-catalysed reactions, micellar catalysis, in aqueous solution at least, has neither the efficiency nor the specificity of enzyme catalysis. Nonetheless, recent work on reversed micelles (that is, those enclosing hydrophilic regions of molecular dimensions in a predominantly hydrophobic environment) suggests that catalysis in reversed micelle systems will more closely resemble that by enzymes.

The authors of this book have contributed a great deal to our knowledge of this topic, and have produced what must surely be the definitive volume for some time to come. The title is perhaps misleadingly restrictive, as the authors have included much informative background material on the structure of micelles and the thermodynamics and kinetics of their formation. A noteworthy feature of the book is the lengthy tables which collate all published information on micellar catalysis up to June 1974. A supplement at the end of the book updates the literature survey to December 1974 Both the authors and the publishers are to be commended on the short time interval between the most recent reference and the publication of the book. This book will be an essential purchase for the library of all laboratories concerned with physicochemical studies on surfactants.

J. E. Crooks

Alkaloid miscellany

The Alkaloids: Chemistry and Physiology, Volume 15. Edited by R. H. F. Manske. Pp. xv+315. (Academic: New York and London, July 1975.) $39.50; £19.75.

THIS latest review volume maintains the style of previous issues with contributions on the ergot alkaloids by P. A. Stadler and P. Stütz and on the Amaryllidaceae alkaloids by C. Fuganti, subjects previously reviewed in volumes 8 (1965) and 11 (1968) respectively. Two chapters consider topics not covered by specific reviews in previous issues. S. Yamamura and Y. Hirata have provided a first review for the series on the Daphniphyllum alkaloids, compounds containing the 2-azabicyclo [3.3.1] nonane system isolated from the Japanese tree, *Daphniphyllum macropodum,* and related species, whereas R. Tschesche and E. U. Kaussmann have compiled a chapter on the cyclopeptide alkaloids, substances on which research has focused within the past decade. This account is primarily chemical and includes a major section on spectral methods, largely mass spectrometry, whereas present biochemical and pharmacological knowledge merits only a page. In contrast, pharmacology and toxicology are the centres of attention in a section by V. Preininger on the Papaveraceae alkaloids other than morphine and codeine, chemical aspects having been reviewed recently elsewhere R. H. F. Manske ends the book with his regular sifting of the journals entitled "Alkaloids unclassified and of unknown structure" (no reference after 1973), including four entries referencing alkaloids from Coleoptera.

This volume will find its main market in the specialist libraries as a most valuable reference work. Its formidable price and the detail of the presentation will deter all who do not possess a special interest in the alkaloids The active researcher in the field, having a good grasp of the primary research literature in his particular area, will probably be most immediately interested in the bibliographies which, however, frequently and necessarily include much which is not recent. Thus, in the chapter on ergot alkaloids, the median date of references is 1968.

The present volume, which admirably maintains the high standards of the series, will constitute necessary reading for scientists of different disciplines who share a common interest in the alkaloids, and should become an important addition to the shelves of many libraries.

E. S. Albone

Muscle receptors

Muscle Receptors. (Handbook of Sensory Physiology, Volume 3, Part 2.) Edited by C. C. Hunt. Pp. viii+312. (Springer: Berlin and New York, 1974.) DM104; $42.50.

THIS volume is made up of three different sections, each by a different author. A long (190 pages) section by Barker on the anatomy of muscle receptors is followed by much shorter sections on their physiology (Hunt) and central actions (McIntyre).

The anatomical section is a very full review of the present state of the subject with numerous illustrations and a comprehensive bibliography; it fills a real need and it will serve as a valuable reference book. The greater part of this section deals with muscle spindles, although other receptors are also very adequately treated. Many aspects of muscle spindle anatomy and its relationship to function remain controversial—and here Barker's own views come across quite clearly—although he takes care to mention the points of disagreement.

In the other two sections of this volume the authors have been in some difficulty: Matthews' very full account of the properties of mammalian muscle receptors appeared only two years earlier, and there was clearly no need for another comprehensive review

Hunt deals briefly with many of the properties of muscle receptors and then gives a more detailed account of some recent work on muscle spindles, and especially on the relationship between the different fusimotor effects, the different types of motor ending and the activity of different intra-fusal fibres. There is also useful information about some non-mammalian species.

McIntyre has written a lucid account of the central actions of impulses from muscle afferents, starting with a useful section on methods of selective activation of afferent fibres. By treating each part of the subject in a historical way he builds up a clear picture of the present position and the present problems. This section is brief and somewhat limited in scope, but within these limitations, it is excellent.

Hunt gives, therefore, useful information on physiological work of the last year or two and McIntyre provides a section that serves as a valuable introduction to the central actions of muscle afferents; but it is the long section by Barker on anatomy that will make many teachers, research workers and advanced students wish to have this book on their shelves.

Peter M. H. Rack

Combating drought

Physiological Aspects of Dryland Farming. Edited by U. S. Gupta. (Oxford and IBH: New Delhi, Bombay and Calcutta, 1975.) RS.80.00.

DROUGHT is the major factor limiting world food production and its occurence is inevitable, but its effects may be reduced by developing a dry farming technology based on an understanding of plant–water relationships. Eleven different authors review aspects of this subject, beginning with a detailed consideration of the physiological principles of dryland crop production by Arnon (Israel). There follow reviews of drought injury and resistance of crop plants by Larson (US), root patterns in crop plants by Hurd and Spratt (Canada), the role of mulches by Unger (U.S A.) and the effects of humidity on crop growth by O'Leary (U.S A.) and of wind by Sturrock (New Zealand).

As almost all the water taken up by the roots is transpired to the atmosphere by the leaves there is considerable interest in the use of anti-transpirants to reduce water losses from the leaves and this aspect is discussed by Davenport and Hagan (US). As photosynthesis proceeds much dry matter is lost through photorespiration and the biochemistry of this process is described by Goldsworthy (UK), who also speculates on the remote possibility of reducing this loss in the future—either by using chemicals or by the development by plant breeders of a wider range of crops with C-4 metabolism which do not seem to photorespire Finally, Iwata (Japan) summarises information on the heat unit concept of crop maturity and discusses its application in crop production. Each chapter is supported by a long list of references to published literature for those wishing to probe deeper

Although the emphasis is on dryland farming the principles discussed apply also to crops grown in more favourable water regimes, and this book has a much wider value to agronomists, crop physiologists, plant physiologists, botanists and ecologists than that suggested by the title.

J. D. Ivins

Fundamentals of neurophysiology

Fundamentals of Neurophysiology. Edited by Robert F. Schmidt. Pp. ix+293. (Springer: Heidelberg, Berlin and New York, 1975.) DM29; $12.50.

THE cost of most reference physiological textbooks has created a demand for less expensive paperback versions. Unfortunately for those who teach neurophysiology these condensed paperback versions usually give an inadequate account of that subject. *Fundamentals of Neurophysiology* is a paperback English edition of a German textbook based on a programmed textbook of a series of lectures to first-year physiology students.

The book is divided into eight sections Those concerned with excitation of nerve and muscle, synaptic transmission and properties of muscle are very good. The sensory system is well presented although there is an inadequate account of the physiology of pain. The sections concerned with reflexes, the motor system and the autonomic nervous system are based on the hypothesis that there is a hierarchical organisation within the nervous system with "higher" and "lower" centres The higher centres control the lower, and together they somehow mould a great variety of stereotyped reflexes into what we know as movement and posture This was a very productive hypothesis in the last century but it has been falsified so often in this century that its use in a modern textbook of neurophysiology is questionable.

After each major topic within a section are a series of questions which the reader is advised to answer before proceeding. This method of self assessment, if it is to be successful, requires careful attention to the content and phrasing of both questions and answers. Unhappily in some sections both the questions and some choices given for answers are trivial

Apart from that and minor points of disagreement which are undoubtedly the result of having to simplify and condense controversial concepts, the authors have written an excellent textbook for most neurophysiological courses The text is well written, the illustrations are unambiguous, and a consistent, adequate standard for the medical physiology student is maintained. Unfortunately the price will put this book beyond the reach of all but the most affluent student.

T. H. Koeze

To flower or not to flower . . .

Photoperiodism in Plants. (European Plant Biology Series.) By Daphne Vince-Prue. Pp. xiv+444. (McGraw-Hill: London and New York, May 1975.) £9.95.

PHOTOPERIODISM is one of the most exciting, and yet one of the most mystifying, aspects of the regulation of plant development. It is an extremely difficult topic to teach, and consequently often receives scant attention in university courses. That is unfortunate, since it offers an almost unique opportunity to study the mechanisms of developmental control. The induction of flowering involves a change from the steady-state, non-determinate formation of vegetative organs at the shoot apex, to a completely new, determinate, sequence of events in which a series of organs, never previously produced by the plant, are formed. Those organs—which together form the flower or inflorescence—although having morphological homologies with the vegetative organ are anatomically and structurally quite different and often produce metabolites and secondary products not synthesised under vegetative conditions. This major redirection of development, inducible merely by a subtle change in the daily length of the dark period, must surely involve, as a causal step, the selective and qualitative regulation of the expression of genetic information. In no other organisms can the whole pattern of organ development be so readily manipulated, and one might justifiably hope that investigations into photoperiodism in higher plants would yield information and concepts of central importance to our understanding of development.

That this happy outcome has not yet materialised, is probably largely attributable to the diffuse nature of our current knowledge of photoperiodism. Many potential investigators must be discouraged by the need to cope with so much apparently unrelated information. What Dr Vince-Prue has attempted to do in this book is to assist the reader to see the wood through the trees. The easy way to do that, of course, is for the author to ignore the great diversity of information, and merely present a personally selected, unified view. Dr. Vince-Prue has eschewed that approach, and throughout the book has unflinchingly provided the reader with the available information, presented in an admirably clear, concise, and, above all, readable form; where logical conclusions can be drawn, she draws them, but she is not unwilling to leave unresolved questions open. In my opinion, that is exactly the right approach, and Dr Vince-Prue succeeds in her formidable enterprise through her immense knowledge and exceptional understanding of the topic.

The book is logically organised and covers all those aspects necessary for a sound understanding. It will prove to be an excellent book for university teachers and research workers, and many undergraduates will find it invaluable as a source-book. Particularly useful are several chapters on the photoperiodic control of development other than flowering.

After reading the book with enjoyment, however, I still find myself impressed more by the diversity of the information available, than by the power of the unified concepts derived from that information. This is a reflection of the subject though, not of the book, and it may be why Dr Vince-Prue has quoted in her Preface a beautifully enigmatic line from Hamlet: "Why day is day, night night, and time is time."

Harry Smith

More than black and white

Race Differences in Intelligence. (A Series of Books in Psychology.) John C. Loehlin, Gardner Lindzey and J. N. Spuhler. Pp. xii+ 380. (Freemen: San Francisco and Reading, June 1975.) £3.00.

THE goal of this book is to consider the available evidence on the relative importance of genetic and environmental variation in accounting for racial–ethnic IQ differences in the United States. The book is divided into three sections. The first considers the background and recent history of the race–IQ controversy and deals with three of the key concepts on which the controversy focuses: the concepts of race, intelligence and heritability. The second section deals with the empirical evidence, and considers a wide number of studies. The final section contains the conclusions and also the authors' views on the implications of these conclusions for the formulation of social policy.

The value of this book is that it provides an excellent summary of the available data on studies dealing with the race–IQ question. But my initial enthusiasm for the book gradually declined as I realised that the authors have skimmed over some of the most important issues. Their discussion of the controversy is far from complete.

The authors consider some of the problems associated with cultural bias in IQ tests and point out that it may be impossible to identify test items "culturally fair" to a particular group. But then they side-step the question of whether the existence of cultural bias in tests makes all attempts at cross-cultural comparison meaningless. But it is when discussing heritability that this book really falls down, as in fact do all arguments concerning race–IQ differences. The authors do not seem to have grasped that fact.

They still subscribe to the belief that a knowledge of the heritability of a trait within a population can tell us something about between-group differences. That is just not so. A measure of heritability is only relevant within the population and environment in which it was measured. No comparison between populations can be made from a heritability measure. It is well known that American blacks are far worse off than whites for a number of environmental factors known to influence performance on IQ tests substantially. These include educational inferiority, lower nutritional levels, cultural biasing in tests, racial prejudice, lack of incentive, to name but a few; as well as the fact of being black in a white dominated society. We have no way of correcting test scores for these systematic differences to obtain unbiased figures. It is impossible to make *a priori* judgements of different aspects of the environment, as every ecologist and plant physiologist knows.

The authors believe that a heritability measure is of use in the present controversy since we are discussing the effect of the modification of environmental variables which are within the existing range of environments. This assumes an additivity of effects, for which there is no evidence. Large differences in traits which are highly heritable can be caused solely by environmental differences. Environmental changes such as the amount of trace elements in the soil can have profound effects on genotypic expression. On the other hand, even if an observed difference is genetic it does not mean that a trait cannot be altered substantially by an appropriate environmental change. An example is the treatment of phenylketonurics with a diet deficient in phenylketonuria. It is meaningless to try and separate genetic and environmental contributions to IQ differences between racial groups.

The authors conclude that some research on differences in ability between US racial–ethnic groups seems to be of fairly high scientific and social priority. It is difficult to see how they can reach that conclusion. From their extensive discussions on the studies quoted the authors have demonstrated that the whole race–IQ question is in fact unanswerable.

Glenys Thomson

Animal mechanics

How Mammals Run: Anatomical Adaptations. By P. P. Gambaryan. Pp. xiv+367. (Israel Program for Scientific Translations: Jerusalem; Wiley: New York and Chichester, April 1975.) £10.80.

GAMBARYAN and his colleagues have assembled a large body of knowledge on terrestrial locomotion which has hitherto been available only in Russian, and little known in the West. They have dissected, and measured the limb bones and muscles of, about a hundred species from seven orders of mammals and filmed nearly half these species running at various speeds. Their data was collected, with data from other sources, and translated into English.

The book includes a large collection of illustrations traced from film sequences and still photographs. There are a great many illustrations of small mammals, as well as of the larger ones which are well illustrated in other books such as Muybridge's *Animals in Motion.* These illustrations are well chosen, and Gambaryan makes good use of them. His comparison of the gallop and ricochet and discussion of the circumstances in which each evolved is particularly interesting.

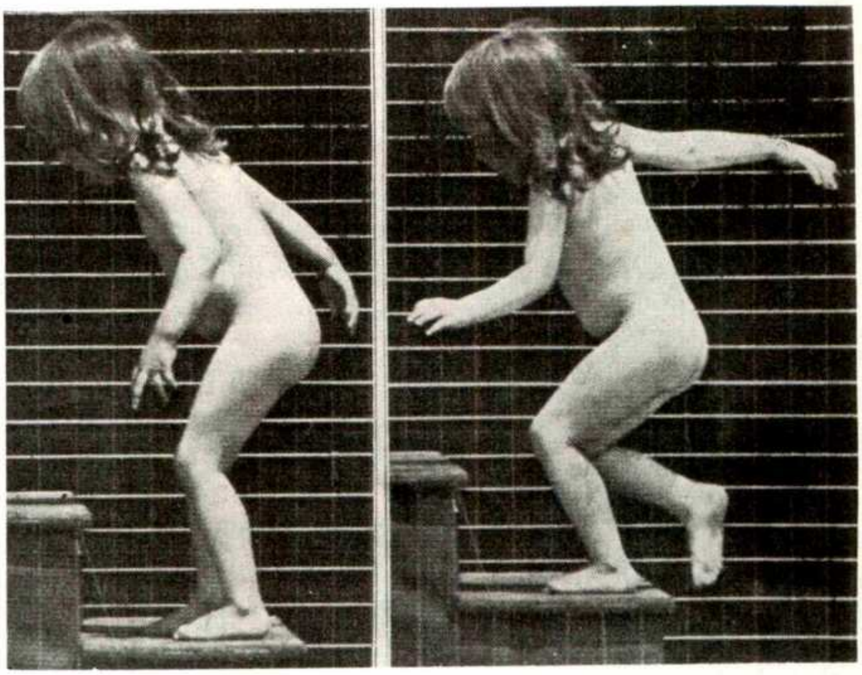
The data on muscle weights is extensive, but of limited use. Gambaryan is well aware of the significance of moment arms, cross-sectional areas and pennation patterns, but tells us little about them.

Recent Western research on terrestrial locomotion has used a wide variety of techniques including light and X-ray cinematography, electromyography, force transduction and measurement of oxygen consumption. The Russian work reported in this book used a more restricted range of techniques. No X-ray cinematographs or measurements of oxygen consumption were made. Electromyography was carried out but only on the cat, duplicating work done in the West. Force platforms were used but they were capable of registering only vertical components of force.

Much of the book is devoted to comparisons between mammals, some of which are most enlightening but others are unconvincing because of lack of rigorous mechanical analysis. Assumptions are made about the directions of the forces exerted by feet which conflict with all force platform records, including those in Manter's paper of 1938 (which is cited). The attempts at theoretical mechanical analysis are more elementary and less penetrating than the analysis in Smith and Savage's paper of 1956 (which is cited). There is no appreciation of the roles of elastic storage of energy or of negative work in running.

This is a valuable book but it is not an adequate account of current knowledge in this subject.

R. McNeill Alexander



Mary Evans Picture Library

One of Muybridge's illustrations

Structural Materials in Animals. By C. H. Brown. Pp. xv+448. (Pitman: London, July, 1975.) £10.50.

STRUCTURAL studies on the fibrous molecules of skeletal systems in organisms were important contributions to the early growth of Molecular Biology. Nowadays it is more fashionable to identify Molecular Biology with the study of enzymes and nucleic acids, and the problems involved in synthetic, metabolic and genetic processes. Happily, the fibrous molecules have not been entirely neglected. Several excellent books on the chemistry and conformation of structural biopolymers have appeared recently. Dr Brown's addition to this literature is a thorough, somewhat laborious, account of how and where these materials occur throughout the animal kingdom. Her book is aimed primarily at zoologists, who will be familiar with the standard textbook approach.

An elementary introduction to the chemistry, conformation and molecular organisation of the main structural molecules prefaces detailed descriptions of their occurrence and incorporation into skeletal structures and tissues in each phylum from Protozoa to Chordata. A short chapter on decay of structural materials is followed by some general conclusions in which an attempt is made to develop a more comparative theme. The style of writing is clear, direct and unpretentious, but in common with many similar catalogues of research results, the narrative is apt to be pedestrian. In general the illustrations are well chosen for their good quality and pertinence to the text, but some diagrams seem to be superfluous. For example, the diagram showing the comparative volume of coelenterates occupied by mesogloea may have done much to clarify the original article from which it has been taken, but in this book it adds little to the accompanying text.

There are a number of trivial factual mistakes, and the text is littered throughout with spelling and printing errors—a deplorable feature which is all too common in scientific volumes nowadays. But the worst feature of this book is the inexcusable and inordinate number of bibliographical errors. So many authors' names are mis-spelled in the text or reference list, and sometimes in both. One particular name is spelled in three different ways in the space of two pages, whereas some others appear in two different versions on the same page. Inattentive proof reading—or none at all—has devalued what is otherwise a useful book. Such errors may not deter the general reader, but for research workers who may wish to consult this book these mistakes will be a constant source of irritation. Furthermore, it is very frustrating when (on at least one occasion) a paper quoted in the text is not listed in the references.

W. Kenchington

obituary

Sir James Wilfred Cook, FRS, sometime Regius Professor of Chemistry in the University of Glasgow and Vice-Chancellor of the Universities of Exeter and East Africa, died on October 21 at the age of 74.

After graduating from University College, London, Sir James became lecturer in chemistry at the Sir John Cass Technical Institute in 1920, where be obtained his PhD on the chemistry of anthracene derivatives. Sir Ernest Kennaway invited him to join the staff of the Royal Cancer Hospital in 1929, where he remained for ten years, becoming reader in pathological chemistry in 1932 and professor of chemistry in 1939. It was at this hospital that he made his outstanding contributions to cancer research, by demonstrating the carcinogenicity of polycyclic benzenoid hydrocarbons and then isolating from coal tar its main carcinogenic component. This work showed for the first time that cancer could be induced by minute quantities of a pure chemical compound. Further work revealed a correlation between structure and carcinogenicity. Cook and Kennaway were awarded the Prize of the International Union against Cancer, and Cook was elected a fellow of the Royal Society in 1938, in recognition for their work. In 1939 he was appointed Regius Professor of Chemistry at Glasgow, a chair which he occupied for sixteen years. While coping ably with his new administrative duties he also found time to act as President of the Royal Institute of Chemistry from 1949–51 and as a member of the University Grants Committee from 1950–54. He continued to work on polycyclic hydrocarbons, and also carried out research in colchicine and the tropolones. In 1954 he became Principal of the University College of the South West and negotiated its conversion to the University of Exeter, becoming its first vice-chancellor in 1955. He was instrumental in bringing about the emergence of a full-blooded university. His honorary directorship of the MRC Carcinogenic Substances Research Unit, at Exeter, helped to keep him in touch with organic chemistry. Cook had always shown an interest in education in developing countries, and on retiring in 1965, became vice-chancellor of the University of East Africa. In 1974 he became Chairman of the Academic Advisory Committee of the New University of Ulster, at Coleraine, and again displayed his enthusiasm in the cohesion of a new venture. He was presented with a knighthood in 1963 and was a Davey Medallist of the Royal Society (1954).

Gustav Hertz, the German Nobel Prize winning physicist, died in East Berlin on October 30, at age of 88.

Dr Hertz, who was a nephew of Heinrich Hertz (renowned for his work on electromagnetic waves) was educated at the Universities of Gottingen, Munich and Berlin. In 1925, he became Professor of Physics at the University of Halle, and in 1928, Director of Physics at the Berlin-Charlottenberg Technische-Hochschule. In 1934, he was obliged to resign from his academic post, since he was unwilling to take the oath of allegiance to the newly-established Nazi regime. From then on, and until the end of the war, he worked for the Siemens company, who built a special laboratory for him. At the end of the war, he went to the Soviet Union, where until 1954 he worked on the Soviet atomic bomb project. He then returned to East Germany, and taught physics at the University of Dresden, until his retirement in 1961. Hertz's Nobel Prize, which he won with Dr J. Franck in 1925, was for work on the ionisation theory of gases, which played a fundamental role in the establishing of quantum theory. During this similar period of his research activity, he also devised a diffusion method of calibrating neon isotopes. His role in the development of the Soviet atomic bomb is difficult to estimate, not only from the classified nature of the project, but also because, as Sakharov has explained, the situation was a complex one of "collective invention". The fact that Hertz was given the Lenin Prize suggests, however, that his contribution was considerable.

Vera Rich

announcements

Awards

Her Majesty the Queen has approved the recommendations made to the Council of the **Royal Society** for the three **Royal Medals** to be awarded as follows: To **E. Bullard** in recognition of his distinction as a world leader in geophysics; to **D. C. Phillips** in recognition of his solution of the three-dimensional structure of an enzyme and his contributions to the techniques of X-ray crystallography; and to **B. Wallis** in recognition of his contributions to aeronautical engineering.

Appointment

A. Alexandrov has been elected president of the Soviet Academy of Sciences.

Reports and publications

Other Countries

United States Department of the Interior: Geological Survey. Bulletin 1395–J: The Supai Group—Subdivision and Nomenclauture. By Edwin D. McKee. Pp. iii + 11. Professional Paper 437–F: Land Subsidence Due to Ground-Water Withdrawal in the Los Banos-Kettleman City Area, California, Part 2. Subsidence and Compaction of Deposits. By William B. Bull. Pp. v + 90. Professional Paper 925: Sedimentation and Tectonics in the Early Tertiary Continental Borderland of Central California. By Tor H. Nilsen and Samuel H Clarke, Jr. Pp. iv + 64. (Washington, DC: Government Printing Office, 1975.) [710

United States Department of the Interior: Geological Survey. Water-Supply Paper 2152: Quality of Surface Waters of the United States, 1970. Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Pp. x + 668. (Washington, DC: Government Printing Office, 1974.) $5.45. [810

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nature

December 11, 1975

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A real forum for scientists

The election of a new President of the Royal Society for a five-year term is an appropriate occasion on which to ask what part Britain's academy of science should play in the scientific life of the nation. The society has been evolving, not only under pressure from outside—it has never lacked vociferous critics—but also under the influence of successive presidents, and it is certainly not beyond its capacity to change even more. But in what direction? Here is one small suggestion.

Rank-and-file Fellows frequently complain that its operations are centred too much on London and are under the control of its Council to an excessive extent. (Those who dispute this latter statement have only to look at the process by which the President is 'elected'.) Fortunately, however, British science as a whole has never seriously suffered from the general tendency to accumulate power and influence in the nation's capital. The strength of the universities has guaranteed that, and however much the bureaucracy might accrete in one place, the intellectual power has been widely scattered.

But universities are not what they were even a few years ago, and the importance of the central bureaucracy is undoubtedly growing. What more imaginative gesture could the Royal Society make in these circumstances than to move some of its activities outside London? These could well include parts of its committee work, but much more generally valuable would be the organisation in the provinces of regular discussion meetings—which need not be major gatherings such as the ones the society has successfully encouraged in the past few years.

There is a need for some forum in which scientists could discuss, at a local level and away from the public gaze, the problems of practising science. Not all of these problems are narrow technical ones; many concern funding, policy, science and society, education and so on. Were the Royal Society to organise regular meetings out of London to discuss these matters, it would be doing a great service in bringing scientists closer together.

Fearless, blunt message to our readers

Our ultimate objective is to continuously assess and ultimately hopefully optimise the degree of clarification inherent in the information-conveying procedure currently selected by scientific practitioners.

At this present time we envision subjecting all written communication to a rigorous and ongoing scrutiny process. The management sees as its mission a systematic evaluation of, or at least an evaluative role in, the determinative processes by which overly extended documentation is submitted to a continuous questioning of its appropriate modalities. Where these do not parallel, or even sub-parallel, currently acceptable norms, the documentation will be the subject of an operationally proven interactive process in which the submitter and a qualified representative of this communication channel group will have as their function the production of a sanitised, re-evaluated word-stream.

To this end, attendees at such encounters should familiarise themselves with the logistics and algorithms currently utilised and should show an awareness of the editorial systems that as of now we have identified as of most central relevance and impact.

The problem-oriented environment to which we address ourselves does not permit us to essentially guaranteee continuous relative stabilisation with respect to our operating procedure paradigms. Future changes may be effectuated when anomalistic situations arise and have undergone assessment; the re-evaluation phase will characteristically be followed subsequently by an implementation of revised and updated procedures and/or systems. Our ultimate objective must always be the optimisation of the two-way information flow, if necessary by innovative support techniques.

We underscore our conviction that the utilisation of word-processing equipment for manuscript generation has to be viewed as a major, not to say a prime, necessity.

At this time the personnel associated with this information-dissemination mode convey to its recipients appropriate greetings for the festive period upcoming momentarily.

Naming the Loch Ness monster

Recent publicity concerning new claims for the existence of the Loch Ness monster has focused on the evidence offered by Sir Peter Scott and Robert Rines. Here, in an article planned to coincide with the now-cancelled symposium in Edinburgh at which the whole issue was due to be discussed, they point out that recent British legislation makes provision for protection to be given to endangered species; to be granted protection, however, an animal should first be given a proper scientific name.

Better, they argue, to be safe than sorry; a name for a species whose existence is still a matter of controversy among many scientists is preferable to none if its protection is to be assured. The name suggested is Nessiteras rhombopteryx.

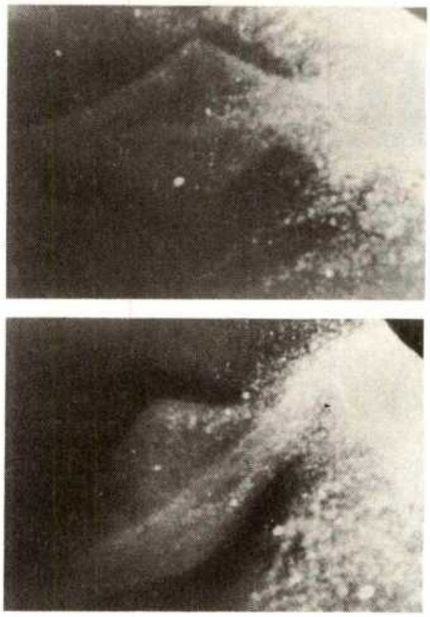

Fig. 1 Photographs taken by strobe flash at a depth of 45 feet in Loch Ness at 0150 h on August 8, 1972, showing the right hind flipper, calculated as about 2 m long, of *Nessiteras rhombopteryx*. The lower picture was taken about 1 min after the upper. The camera was stationary and aimed horizontally. The photographs were taken with equipment devised by Professor Harold Edgerton of the Massachusetts Institute of Technology and have been computer enhanced at the Jet Propulsion Laboratory, Pasadena, California. (Copyright, Academy of Applied Science, Boston, Massachusetts.)

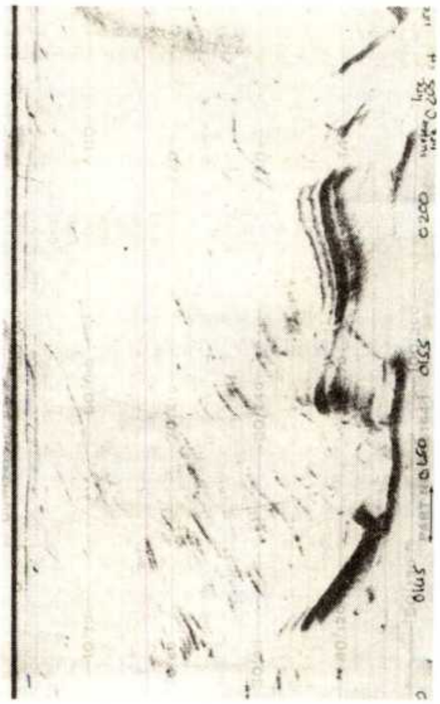

Fig. 2 Sonar trace (Raytheon DE 725 C) of the period when the photographs in Fig. 1 were taken. The sonar set was aimed horizontally, and the strong echoes are at a range of about 40 metres. Sonar frequency 200 kHz. Time marks on the right of the picture are at 5 min intervals. The arrows mark the period during which the photographs were taken. The indications are that two large animals were present.

SCHEDULE 1 of the Conservation of Wild Creatures and Wild Plants Act, 1975, passed recently by the UK Parliament, provides the best way of giving full protection to any animal whose survival is threatened. To be included, an animal should be given a common name and a scientific name. For the Nessie or Loch Ness monster, this would require a formal description, even though the creature's relationship with known species, and even the taxonomic class to which it belongs, remain in doubt.

On August 8, 1972, a team from the Academy of Applied Science, Boston, Massachusetts, working in conjunction with the Loch Ness Investigation Bureau of London, obtained what seems to be the most precise evidence on which to base such a description.

Two consecutive underwater photographs (Fig. 1) were taken by a stationary time-lapse camera with strobe flash, operating automatically at a depth of 45 feet in Loch Ness, along with a simultaneous sonar trace (Fig. 2). The photographs have been computer enhanced at the Jet Propulsion Laboratory in Pasadena, California, a technique which can 'improve' the image by comparing adjacent grains electronically so as to remove haziness, but cannot alter shapes or otherwise falsify the record.

A black triangle in one corner of the photograph is caused by the edge of the strobe flash apparatus, and should be disregarded. The pictures show a flattened, diamond-shaped fin, flipper or paddle, in which the limb structure is not quite central. Calculations from optical data corroborated by simultaneous sonar recordings suggest that the paddle is about 2 m long. Given its function, the 'main spar' of the paddle is likely to be nearer the leading, rather than the trailing edge, suggesting that it is a right-sided paddle.

A neck would be likely anterior to a forelimb, and a wider body posterior to it; since the opposite appears to be the case the photographs are assumed to show a right hind limb. The strobe light illuminates an area of the animal's back and belly with a rough skin-texture. In the upper photograph there is what may be some suggestion of ribs.

Although these two photographs of the hind flipper are the main basis of the description, and the flipper-length is thought to be some 2 m, it is possible, using the evidence from other photographs and from sightings, to indicate some further features and dimensions of the animal. A total body length of 15–20 m seems possible including a neck of 3–4 m with a rather small head which may have some horn-like protuberances. Moving-target-discriminating sonar displays have provided body length measurements of the order of 15 m, and the underwater automatic strobe photography has provided support for the reports of a long neck.

Frequent descriptions liken the back to 'an up-turned boat', and both still photographs and films show this configuration. Further underwater photographs taken in June 1975 may show other aspects of the same species, including a view of the head, neck and body (Fig. 3). The Loch Ness monster may possibly resemble the impression shown in Figs 4 and 5.

It is proposed that the large animal species living in Loch Ness be called *Nessiteras rhombopteryx*, Scott and Rines (nov. genus and species; the only species is automatically the type species) with the common names: the Nessie or Loch Ness monster. The generic name *Nessiteras*, a neuter noun, is a composite word combining the name of the Loch with the Greek word *teras*, genitive *teratos*, which was used from Homer onwards to mean a marvel or wonder, and in a concrete sense for a range of monsters which aroused awe, amazement and often fear. The specific name *rhombopteryx* is a combination of the Greek *rhombos*, a diamond or lozenge shape, and the Greek *pteryx* meaning a fin or wing. Thus the species is the Ness monster with diamond fin.

In trying to determine which class

they belong to, it is concluded that the paddle in Fig. 1 must belong to a vertebrate animal. It has been established that no living cetacean has such a flipper, and the inclination is to view it as reptilian. Further designation must be pure speculation.

It is clearly unsatisfactory, from a zoological point of view, to base a name on photographs rather than on the remains of an animal, or at least some part of it. This means that, for the time being, there is no 'holotype' or 'type-specimen'. But description from an illustration is permitted by the International Code of Zoological Nomenclature, and the procedure seems justified by the urgency of comprehensive conservation measures.

The name proposed does not link the species to any animal or group of animals known to science. It applies to the animal first recorded in Loch Ness at the time of St Columba's visit in 565 AD and perhaps also to similar animals reported in other freshwater lakes in Scotland and elsewhere. *Nessiteras rhombopteryx* probably also has affinities with some fossil marine forms and perhaps also with living 'sea serpents' which have been named in the past. In 1817 Rafinesque named the Massachusetts Bay sea serpent *Megophias monstrosus*. In 1892 Oudemans changed the name to *Megophias megophias* and in 1958 Heuvelmans proposed *Megalotaria longicollis* for the animal sighted in the surrounding seas. We see no reason to suppose that this animal is conspecific or even congeneric with the animals in Loch Ness, and we do not therefore think the names used by Rafinesque, Oudemans and Heuvelmans apply to the owner of the hind flipper in the photographs.

The 'population density' of the Nessies is no doubt dictated by the size of the loch and the abundance of food. Loch Ness is 24 miles long, up to 1.5 miles wide, and 700 feet deep over much of its length, with a deepest point so far discovered of 975 feet. The surface is 50 feet above sea level. Salmon, sea trout and elvers running up the River Ness into the loch can thereafter swim up a number of rivers which run into it, and salmon, sea trout and well grown eels must descend these rivers into the loch on their way back to the sea. There are also resident populations of brown trout, char and sticklebacks. The shallow waters are well grown with freshwater weeds, and organic detritus must also be considered as a possible food source.

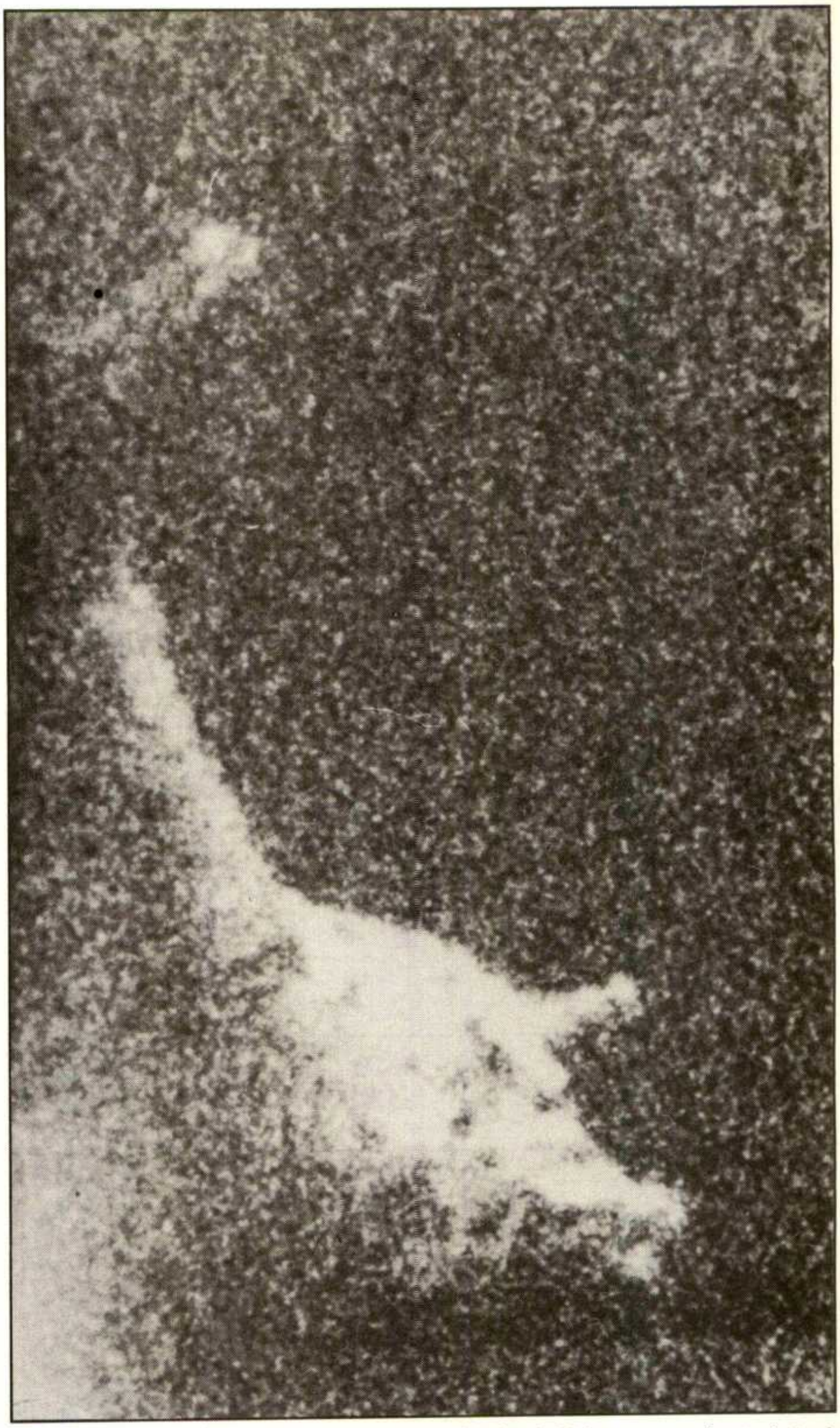

Fig. 3 Photograph taken by strobe flash at a depth of 35 feet in 80 feet or more of water in Loch Ness at 0430 h on June 20, 1975, showing the head and neck (7-12 feet in length) together with part of the body, with appendages, of *Nessiteras rhombopteryx*. The main body structure is 20-25 feet from the camera which is pointing about 30° above horizontal. Adjacent frames, taken about 1 min before and 1 min after, show nothing.
(Copyright, Academy of Applied Science, Boston, Massachusetts.)

It has been calculated that the biomass of the loch could support a population of large animals. Between the melting ice and the present time the loch was probably, rather briefly, an arm of the sea. A population may therefore have become landlocked some 12,000 years ago. The possibility that juvenile animals may have ascended the 5 miles of the River Ness more recently seems less likely. Viable populations of vertebrate animals have been able in a number of cases to survive with less than 30 individuals for considerable periods. It is suggested that there may be a viable population of *Nessiteras rhombopteryx*.

Reptiles must breathe air, though comparatively infrequently. A terrapin has been recorded as surviving one year of continuous submersion. Should the Nessies wish to breathe quite frequently, they would not be detected easily if the nostrils were at the topmost point to break surface. Many accounts of head sightings speak of 'horns' or 'ears' which may be extensions of the nostrils into breathing tubes. Indeed the ancient name 'water-horse' suggests the appearance of horses' ears. With any ripple on the water it would not be difficult for a Nessie to breathe undetected. In flat calm conditions, the surface is constantly dimpled by rising fish, and again the animal would be likely to go unnoticed.

Further attempts to obtain strobe-flash or other underwater photographs with coincident sonar charts may soon lead to a more complete knowledge of the anatomy of *Nessiteras rhombopteryx*. Another technique which could produce results is sonar-linked underwater television which could be recorded on videotape. Efforts might also be made to dredge on the bed of the loch at points where penetrating sonar may indicate more solid objects lodged in the sedimentary mud, with the possibility of finding some recent bones.

Modern concepts of conservation suggest, however, that in the case of very rare animals photographic evidence should perhaps be more frequently used by taxonomists. The ethical implications of collecting specimens of a species in danger of extinction have been raised by certain recent examples. To what extent, if any, does scientific curiosity justify risking that most irrevocable biological occurrence—the extermination of a species? The outcry that would now be raised by any attempt to kill a Loch Ness monster for any purpose whatsoever can well be imagined.

Meanwhile tribute should be paid to all those who have worked, over many years, to identify the Nessies. They have produced a hard core of evidence indicating the general appearance of the animals. Now that their existence seems closer to being established, giving the species a name will not only provide it with the necessary protection but also focus greater attention on further studies which must in due course lead to more detailed knowledge of the animals' anatomy, biology and phylogeny. ☐

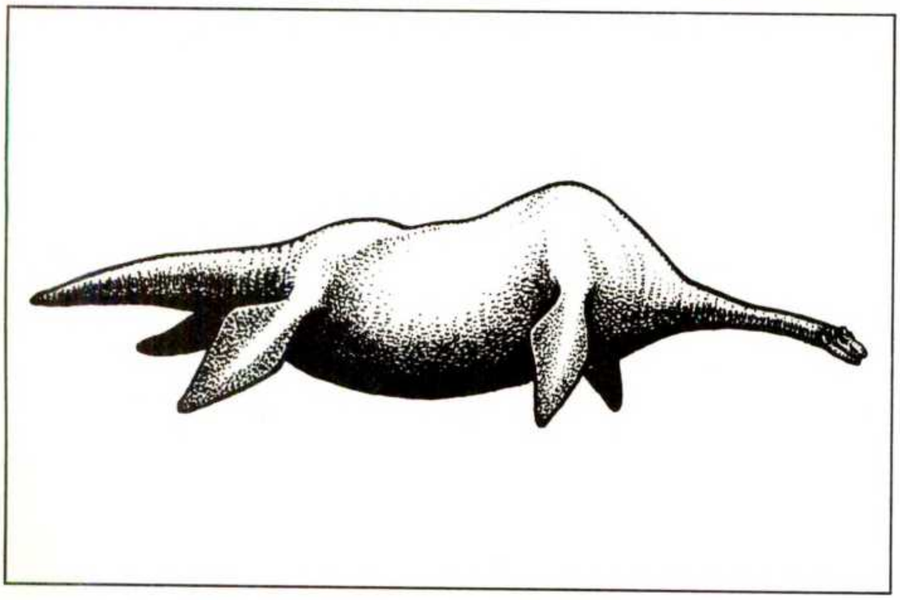

Fig. 4 Impression of the possible appearance of *Nessiteras rhombopteryx* from photographs, eyewitness accounts and sketches derived from a large number of sightings at the surface and from the accompanying underwater pictures.

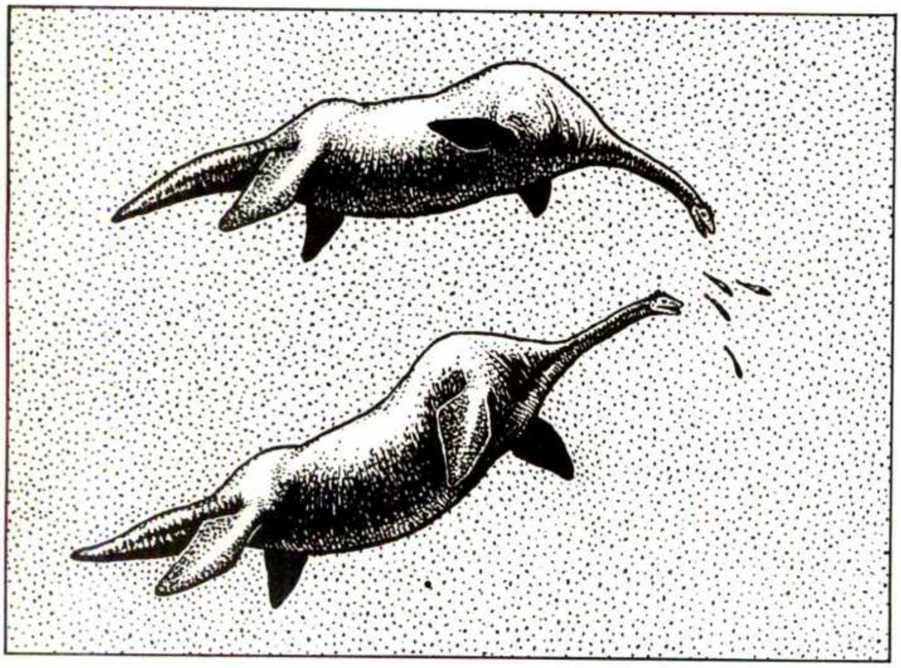

Fig. 5 The food of *Nessiteras rhombopteryx* is probably mainly fish, including salmon.

THE latest claims for the existence of the Loch Ness Monster have their origins in a series of photographs obtained by a team led by Robert Rines, a prominent Boston lawyer. Dr Rines has looked for Nessie every summer since 1970, and two sessions have yielded results. The first was in 1972, when the two 'flipper' photographs were taken underwater. Last June photographs were taken which are now being offered as evidence for the presence of the monster.

The development of two thousand underwater shots, taken automatically at regular, short intervals, revealed six frames with a discernible image, of which two are believed to show the monster. One of these, reproduced on p467, is claimed to show the head, neck and body. The other is claimed to be a close-up of the head.

Dr Rines consulted naturalist Sir Peter Scott, who has fostered an interest in the Loch Ness Monster since 1962 through the Loch Ness Information Bureau. They decided that the evidence should be presented to an invited audience of scientists at a meeting in Edinburgh on December 9 and 10 organised by the Royal Society of Edinburgh, the University of Edinburgh and Heriot-Watt University. This was to be followed by a presentation to the House of Commons.

On November 22, news of the latest claims leaked to the press. There followed a series of statements attributed to some of the scientists who had already seen the photographs. By December 1 so many people had stated their opinions in advance that the planned symposium was cancelled on the grounds that useful or impartial discussion would no longer be possible. The presentation to the House of Commons is to continue.

Microscopic blind spots

No newspaper columnist would expect to claim that 'spiders are insects' or that 'Mars is a star' without attracting a regular deluge of indignant correspondence. Yet even our most respected publications can state that typhoid is caused 'by a virus' and emerge unscathed. Brian Ford comments.

SOCIETY has a blind spot for micro-organisms. Such scientific devices as an automatic transmission system, a programmed washing machine, an electronic calculator or a sophisticated high-fidelity amplifier are familiar features of today's domestic landscape. People are equally familiar with the sights and sounds of science. The craters on Mercury or the boulders on Venus take space of their own in the press, along with integrated circuits, steel-piercing lasers and thermographs. And the hissing whistle of a transmitting satellite is as familiar to our ears as warbling whales and echoing, ghost-like bats.

The microscopic universe, however, remains obscured, and for all the undoubted familiarity of the microscope as a symbol of scientific endeavour there is ample evidence of the esoteric complexity surrounding the subject. Superstitions about infection persist a century after the popularisation of the germ theory, and though they are intimately connected with us, microbes are unfamiliar to the public.

Critics of modern microscopy argue that professional standards of routine technique are inferior too. Certainly one hears far more from research workers who claim to have a love–hate relationship with the instrument than suggestions that microscopy is enjoyable. The blind spot that exists within the discipline is revealed not only by a low average standard of practical microscopy, but also by the paucity of educational opportunities for those who wish to improve. This is vividly reflected in the way we have delayed setting up safety codes for the handling of pathogenic bacteria.

It is perhaps a legacy of nineteenth-century bacteriology that we tend to associate 'microbes' with 'bacteria', and 'bacteria' with 'disease'. It is still accepted that microbiology and bacteriology are virtually synonymous. This tradition of distaste, in might be argued, underlies our microbiological apathy, which has delayed progress in a field rich with potential.

During the debate on energy sources, for example, much was said about waves, windmills and the power of the tide. But little was heard of the solar-powered electron pump systems of algae and the capacity of micro-organisms to act as transducers. The brand of automotive fuel sold as 'Cleveland Discol' was possibly the last one available containing fermentation alcohol, and it is somehow intriguing that the industrial liberation of metabolic energy from a 'waste' substrate is now —in an energy-seeking world—so rare.

Corynebacterium simplex is familiar as the organism which converts cortisone to prednisone and hydrocortisone to prednisolone, both more potent yet less hazardous to the patient than the parent steroid substrate. An extension of cell technology has for years offered us a range of similarly self-fuelling conversions that demand no energy supplies. Yet microbiological methods seem to be used only when an acceptable chemical–industrial approach cannot be found. Should we not reverse the process, and opt for cell technology wherever we can?

The public health field offers limitless possibilities. The antibiotic era has revealed many of the growth-regulating products of the fungi, but such familiar algae as *Microcystis* and the abundant *Chlorella* secrete bacteriostats. Little is known about the ecological importance of microbial interrelationships, yet since the 1930s it has been known that soil-borne pathogens such as *Helminthosporum* and *Ophiobolus graminis* are controlled in some way by micro-organisms in the rhizosphere. Intracellular bacteria can be found in many species of insects (including *Periplaneta,* the cockroach) which will not mature normally without them.

Some instances of overwhelming skin infections in man may be due not to the removal of commensals and the opening of a niche, but rather to the absence of specific health-promoting species—salugens (from the Greek *salus*—health) as opposed to pathogens.

Becoming microbe-conscious opens many avenues of investigation. For all our exhaustive knowledge of the behaviour of a single cell of *E. coli* or *Paramecium,* we know little about the interactions of five cells, or five hundred. The trend towards bulk *in vitro* methodologies is unlikely to resolve these issues unless it is complemented by that rare art, the microscopy of living cells.

A similar philosophical approach allows one to resolve the conflict of the 'cell-equals-egg' *versus* 'cell-equals-organism' debate. This involves demonstrating that a concept of functional delegation can relate the specialisation of cells within a multicellular organism to the complexity of such single-celled organisms as the protozoan *Epidinium,* a rumen protein converter which possesses a brain-like motorium and a segmented skeletal plate and has an almost quasi-chordate appearance. Certainly one can argue that the loss of delegative function in malignancy allows the cancer cell to return to a less specialised, microbe like state (and the world-wide infection of cell cultures with HeLa suggests that, even in the practical sense, that analogy holds true).

The philosophical consequences of becoming microbe-conscious range from the lighthearted concept that man is a free-swimming protozoan called a sperm, and all the complexity of an adult human is nothing more than a refined fruiting-body; to the serious and pragmatic possibility of producing crops, food and drugs from tissue culture rather than from colonies of the entire species.

But whatever the future, there can be little doubt that our orthodoxy has done a disservice to micro-organisms. The dogmatism which followed the Pasteurian view of infection must be replaced with something more realistically positive. For philosopher and technician alike, the benefits of doing so are great.

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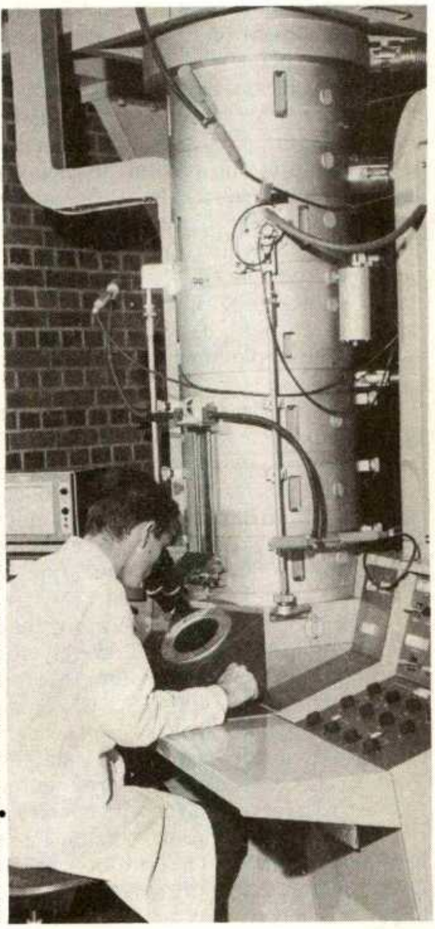


Five million volt electron microscope (Photo: Fox photos)

international news

Fast-breeder urged

by Allan Piper

THE UK standing Royal Commission on Environmental Pollution, which has had the radiological safety aspects of Britain's nuclear programme under consideration since the beginning of last year, has conceded that there is a case for establishing a full-scale demonstration power station based on the controversial fast-breeder reactor (FBR). In an open letter to the Prime Minister, the commission's chairman, Sir Brian Flowers, acknowledged the importance of the FBR in Britain's energy strategy and admitted that "there are arguments for pressing ahead [with a demonstration FBR] on a reasonable time scale. To do so is to be better prepared".

Sir Brian's letter was apparently prompted by fears that the British government would reach an early decision to proceed with a commercial-scale reactor in collaboration with other European governments before the commission's investigation was complete. A demonstration FBR, "remotely sited [with] its own fuel reprocessing and fabrication plant", is seen as vital for assessing the hazards of operating a commercial fast reactor. The letter does make the point, however, that the UK need not allow itself to become finally committed to the FBR at the expense of research into other energy sources. It notes that the commission is not "yet persuaded that the energy needs of the UK in the next 30–50 years are such as unavoidably to require the deployment of FBRs on a massive scale."

Were it not for the apparent paradox of an environmental watchdog body calling for the construction of a controversial type of reactor, the publication of Sir Brian's text might have been the non-event of the year. Not only is the primary recommendation of the letter couched in predictably reserved terms, it is also little more than a reiteration of what is effectively the policy of the United Kingdom Atomic Energy Authority (UKAEA).

For some time it has been widely accepted that the next logical step in the UKAEA's development programme for fast reactors would be the construction of a demonstration reactor on a commercial scale. The prototype 250 MW reactor at Dounreay seems just about to have survived its bout of teething problems, but the UKAEA's tentative plans for the construction of a 1,300 MW demonstration installation nonetheless involve time-scales that seem surprisingly short. The authority has talked of having a demonstration fast-breeder by the early 1980s, though the Department of Energy's decision will depend on prospects for European collaboration.

It is not altogether clear why the text of the letter should have appeared at this particular time, especially as the Prime Minister denied in his reply that an early decision on the FBR programme was expected. Sir Brian and the Prime Minister shared a platform on the day after the publication of the letter, the key passage of which highlighted "the distinction between the environmental implications of a full-scale demonstration FBR station and of a large ongoing nuclear programme". What that means is that the construction of a UKAEA demonstration reactor, which would be only nominally commercial, might be taken to signal a total commitment to the FBR, whereas the construction of the same reactor in line with the commission's recommendation can be viewed as part of an environmental appraisal. □

Protest over Sakharov

from Vera Rich, London

THE award of the 1975 Nobel Peace Prize to Academician Andrei Sakharov has inevitably drawn considerable attention to the whole problem of human rights and academic freedom in the Soviet Union. Sakharov was refused a visa to travel to Oslo to collect his prize, and a group of former Nobel winners has now issued a statement on behalf of the refusnik scientists "deprived of the freedom to work, to publish, to teach, and to travel freely, simply because they have sought to emigrate from the USSR".

They note that "we shall never regard scientific exchanges with the USSR as being easy and normal while these people are deprived of their rights . . . while arbitrary and needless bars to freedom and work are enforced and while bargains are entered into and unilaterally set aside, the existence of detente will remain precarious". The "bargain" referred to is the one which the Soviet authorities made last year with Academician Veniamin Levich, whom they promised would have permission to emigrate at the end of 1975 when his security restriction expired. In October they denied that any such promise had been made.

Signatories to the statement include Professor Sir Derek Barton, Professor Sir Hans Krebs, Professor Rodney Porter, Professor Sir George Porter and Professor Max Perutz.

Meanwhile, Dr Leonid Kantorovich, joint winner of this year's Nobel Prize for Economics, arrived in Stockholm to receive his prize with the necessary exit visas and approvals demanded by the Soviet authorities. He declined to comment on their refusal to allow Academician Sakharov to travel to Oslo. □

Soviet grain estimate cropped

from Vera Rich, London

DURING this year, the estimates for the Soviet grain harvest have been consistently revised downwards from the planned yield of 215.7 million tonnes announced last spring. The final figure has, however, proved to be even lower than expected. In a statement made to the Supreme Soviet on December 3, Grigorii Vashchenko, Chairman of the Planning and Budgetary Commission, said that the average annual grain production for the ninth five-year plan (which ends this year) had exceeded that of the previous plan by 8%.

Comparative figures are a classical gambit of Soviet planners who wish to put the best possible political face on a deficit. Translated in real terms, however, Vashchenko's 8% "increase" corresponds to a total production of some 137 million tonnes, more than a quarter of the planned output. This figure, which received wide publicity in the Western media, was promptly denied by the foreign service of Moscow Radio. Moscow Radio claimed that the figures had been manipulated and that, properly understood, they reflected a "steady planned progress". In a similar way, the industrial growth rate of 4.3% for the coming year—the lowest since the Second World War, almost certainly as a result of the disastrous harvest—was defended by the Hungarian service of Moscow Radio as marking a "very great step forward in a people's economy like the Soviet Union".

The reason for the great deficit in grain production was attributed by Mr Vashchenko to "difficult weather conditions in several areas of the country". This would seem to put the responsibility firmly beyond the competence of the planners, but this needs further clarification, for the expansion of Soviet agriculture, as the Virgin Lands were opened up, itself had unfavourable climatic consequences. Moreover, an assessment of the agricultural situation based on land resources, available man- and tractor-power, present capacity of irrigation channels and so forth might well reveal that, allowing a reasonable margin for losses due to adverse weather, the Soviet Union is not fully able to feed its population.

This is true of many countries, but these mostly have agreements with countries in surplus to make up the shortfall. This the Soviet government is reluctant to do, and any such negotiations—such as those with the USA—are considered "short-term" solutions to an immediate emergency. A regular deficit would throw into question the whole system of socialist planning.

In his address to the Supreme Soviet, Nikolai K. Baibakov, Chairman of the State Planning Commission, said Soviet agriculture would have at its disposal in 1976 "about 380,000 tractors, 270,000 heavy and specialised trucks, more than 97,000 harvesters, and 78.6 million tonnes of fertiliser and 'food additives'." Agriculture was to be considered as simply another branch of industry, and re-equipped accordingly. Similarly, Mr Brezhnev in his address noted that "in order to raise the well-being of the people, it is necessary to pursue in the future a policy of intensification in agriculture. To turn it into a highly developed branch of the economy demands the systematic consolidation of the material and technical bases of agriculture and the increasing utilisation of the economic and scientific-technical potential for this purpose. Under the Tenth Five-Year Plan, it is intended to allot greater capital investments to agriculture, and to increase supplies of equipment, machinery, and mineral fertilisers."

This "more-of-the-same" approach seems to contrast with the hint of change contained in *Pravda* on November 14. It published a significant decree of the Central Committee of the Party, which was subsequently followed by extensive press comment. This decree approved and recommended for emulation a project undertaken by the Lithuanian Agricultural Research Institute, on the improvement of the efficiency of basic research in agriculture and its implementation in practice. The obvious nature of what the Lithuanian agronomists have done makes this surprising. The project, which has lasted eight years, consisted of research into soil types, problems of land reclamation, and the productivity of various strains of pasture and human and animal feedstuffs. Soil charts were compiled, and the yield of reclaimed soils were closely examined. This raises the question of what the real basis of Soviet agricultural planning has been. Hitherto it seems to have been considered sufficient to mechanise and "chemicise" agriculture, in the hopes that a good yield must surely follow. The emphasis placed by the Party on the Lithuanian project seems to show that in spite of public pronouncements on the need to introduce industrial methods into agriculture, there is a growing awareness of the need to base all such plans on a study, not only of general climatic and geographical conditions, but also on local micro-ecologies. □

Another look at doom

The main trouble with the computer simulations of environmental change that are used for long range forecasting—such as those used by the Club of Rome to compile *Limits to Growth*—is, according to Lord Ashby, that they omit politics, taking no account of the most striking and distinctive feature of all communities of organisms: their homeostatic response and capacity for adaptation.

What is needed, he argued when he delivered the 21st Fawley Foundation Lecture in Southampton this week, is "a second look at doom": a recognition that the prognosis may be wrong but the malady—a climacteric rather than a crisis—remains. The arrival at the second turning point in the S-shaped trends of such factors as population and consumption of resources, he said, provoked "political and even ethical responses from society", as well as the more familiar negative feedback effects tending to reduce population or the rate at which resources are used. To ignore this, he suggested, was to fail to grasp the problem.

For Lord Ashby, a well-known biologist who was Master at Clare College, Cambridge, the essential problem was clear. He saw no guaranteed tenure for man on earth—indeed, "it would be an evolutionary anomaly if *Homo sapiens* were not to go the way of the pterodactyl", and a "historical anomaly if western technological man's economic and social system were not to go the way of the cultures of the Minoans and Aztecs". But at the turning point of the S-shaped curves, values changed and deeply embedded social attitudes were reversed. This had already been revealed in UK population trends, in the consumption of energy, and even in such fields of pure science as bacterial genetics.

Exhortations to control the use of material resources and protect future generations, he therefore contended, emphasised the wrong priorities for policy-makers. The imminent danger was collapse due to political and social disintegration. Moreover, the urgent need was to do something about the communities already suffering from the perils forecast.

The main problems were neither technological nor economic, but geopolitical. With the industrialised nations dependent for some essential supplies on underdeveloped and sometimes unfriendly countries, the world was in for a succession of geopolitical confrontations, in which an alliance among nations which owned resources might be directed against nations which needed them. A shift might occur in the balance of world power to which the industrialised countries had to reconcile themselves or else face the alternative of war.

It was not only international tensions which might pre-empt the factors foreshadowing economic collapse described in *Limits to Growth*. Unresolved tensions within nations also revealed the problems stemming from what Lord Ashby described as the instability of man-made eco-systems. A pre-occupation with population control, or resource conservation, or pollution might divert attention from the industrialised nations' new dependence and their desire to maintain their liberal democratic forms—a prospect which, he argued, meant ignoring more imminent and greater dangers. □

Wilson on Blackett

PRIME MINISTER Harold Wilson was in reminiscent mood when delivering the Blackett Memorial Lecture and naming the Physics Department the Blackett Laboratory at Imperial College last week. Patrick Blackett had been professor of physics at Imperial from 1953-65, and had worked with Harold Wilson since the late 1940's, first as Wilson's appointee on the National Research Development Corporation (NRDC), on the board of which he served from 1949-64, and later as Scientific Adviser to the Ministry of Technology in the 1964 administration.

The appointment of Blackett to NRDC, Wilson said, led to one of the most violent political reactions he had known in 30 years of Parliamentary life. Blackett's unorthodox views on world affairs, including the need to co-operate with the Soviet Union to avoid the division of the world into nuclear blocs, was the cause of much of this reaction. But he made a major contribution to NRDC, and in the late 1950's and early 60's, with Labour out of office, his experiences at the corporation had a powerful influence on his thinking about how the next Labour government might improve links between science, technology and industry. The deliberations of such as Snow, Bernal, Blackett, Carter and Bronowski helped lead to the Ministry of Technology which was in some ways NRDC 'writ large'.

It was Blackett, Wilson continued, who, when Mintech was formed, warned of the British computer industry's distress. Supportive action had to be taken within weeks, and Cabinet took a lead by reviewing all proposed departmental purchases of foreign computers. This soon cut down enthusiasm for imports, although there was probably, he added, a good PhD thesis for someone in studying how this new form of protectionism had fitted in with GATT. □

Paying for pollution

IN a Hobart paper published this week by the Institute of Economic Affairs (£1), Dr Wilfred Beckerman has refined his case in favour of charges as a method for controlling pollution and reinforced his criticisms of the alternative method of governmental regulation.

Dr Beckerman, who is a member of the UK's Royal Commission on Environmental Pollution, originally made his points with Lord Zuckerman in a minority report of the Commission in 1972. He suggests that production "uses up" the environment as well as labour and raw materials. With the environment a "scarce resource", the rational objective of "optimal pollution" is to be achieved using the means accepted for the allocation of labour and raw materials.

The polluter, he contends, has to be induced to economise in "using up" the environment, and encouraged to discover means of reducing it through the price mechanism. This, Dr Beckerman suggests, would be as efficient and equitable a method as any, and would also be cheaper. In addition "cosy relationships" between polluters and officials would be circumvented. □

French research body branches out

from the Staff of La Recherche

ONE of France's major scientific research bodies, the *Centre Nationale de la Recherche Scientifique* (CNRS), has recently been taking steps to fulfill more adequately one of the roles assigned to it by its constitution, which states that it should carry out research which will be of direct benefit to the advancement of science "or to the national economy".

The institution has established an industrial relations committee to coordinate a policy of cooperation with industry which it has launched, and is also making strenuous efforts to encourage its researchers to consider the socioeconomic impact of their work. Alongside the traditional scientific disciplines—physics, chemistry, biology—two new branches have been created: science for the engineer, and an interdisciplinary research programme on solar energy.

In addition, the CNRS has signed agreements for the exchange of research workers with several public or semi-public companies including the oil company Elf-Erap and the French Petroleum Institute. It has also reached an agreement on scientific collaboration with the big private chemical company Rhône-Poulenc (R-P) which allows collaboration in all the major fields covered by the company or its subsidiaries. These include organic chemistry, textiles, biology, and toxicology.

Under the latter agreement, a committee with equal representation from R-P and CNRS will choose projects for joint research and organise the training of research workers. Researchers from CNRS, while retaining their salary, will be seconded for one month to the R-P laboratories, and *vice versa.* It is also hoped to involve some personnel from R-P in various committees of CNRS on which industrial representatives could serve. In order to maintain the confidentiality necessary to industry, one clause requires that "no confidential information may be passed on to a third party . . . for fifteen years". Nevertheless, researchers can publish if they have authorisation from the joint committee. If a new process should be invented through this cooperation, R-P, if it wishes, can be the sole beneficiary. Payment to the CNRS would be calculated appropriately.

The terms of the agreement, only recently made available to researchers, trades unions and journalists, have caused an outcry. This has surprised the signatories, for whom the agreement merely makes official the links which already exist between certain CNRS laboratories and the company. They had hoped it would serve as a model for agreements with other companies: the next one is expected between the CNRS and a giant in the French chemical industry, the Péchiney-Ugine Kuhlmann company.

According to its directors, the CNRS does not commit itself to anything, since in principle every researcher is free to accept or refuse to collaborate with R-P. But for many scientists the basis of the agreement is debatable. The advantage to R-P is obvious, in that they will have the use of research workers trained and paid by the CNRS. For the CNRS, the advantage is not so clear; indeed, the agreement appears to be a gift from the public sector, not to industry as a whole, but to one privileged member. Whether or not the research workers, traditionally chary of collaborating with industry, finally feel reassured, they will have great difficulty in publishing their results, often with a delay of a year, and sometimes 15 years.

Of course, the board of directors of CNRS could be apprised of their work. How that information could be transmitted to the scientific community, or to the committees which decide promotion steps in a research worker's career, remains unclear. However, contrary to the practice in other countries, there is very little important high level research going on in industrial laboratories in France, and a period of instruction within a firm should lead, according to the thinking of the CNRS directors, to a mobility of researchers and so free CNRS posts. At present this happens only rarely.

The manner in which the agreement was made is also regarded by many as questionable. There was no consultation, or prior warning to either the commissions (made up of researchers and elected members), or to the other bodies controlling the CNRS (such as the Directorate, or the Administrative Council). But the CNRS apparently intends to push its researchers to cooperate with industry, and to reorientate its research programmes to be more directly applicable.

The idea is not a bad one in principle: recent meetings, when industrial researchers explained their problems to the more basic research workers, have shown that dialogue is possible. It is believed essential, however, that the CNRS should profit from the agreement it makes, and that it should ensure that the results of research financed by the nation are evenly shared and exploited by the nation; this is not seriously safeguarded in the contract with Rhône-Poulenc. Moreover, the more applied research must also leave room for fundamental research.

Fight for the falcon

from Wendy Barnaby, Stockholm

THE Peregrine falcon is in imminent danger of dying out in Sweden. At the beginning of this century there were an estimated 1,000 pairs, chiefly in the north, the west and the region west of Stockholm. By 1945 the number was reduced to 350 pairs, by 1965 to 35, and by 1974 to nine. This year only six pairs are left.

The falcons have declined in the past 50 years mainly because of the widespread use of pesticides, especially heavy metals such as mercury and chlorinated hydrocarbons. Along with this, hunters (particularly the owners of carrier pigeons) have systematically shot the birds and robbed their nests. The Peregrine did not become a protected bird in Sweden until 1957.

All of the information known about the plight of the falcon in Sweden has been compiled by the Swedish Society for Nature Conservation (SNF), a non-governmental, non-profit organisation with about 60,000 members. In 1972 the SNF began a project to make annual inventories of the bird and to study the reasons for its decline. This year expenses for the project amount to Sk100,000 (about $20,000), which is provided by various private funds, the Swedish branch of the World Wildlife Fund, the Swedish Environment Protection Board and the SNF itself. As well as inventories, the programme includes studies of the falcons' food analyses of pesticides and the protection of nests and nesting areas.

The project leader, Mr Peter Lindberg, has surveyed the falcons' prey to determine the regularity with which certain species are consumed, but the results have not been very illuminating because little is so far known about the ways in which these species are contaminated with pesticides. Nor has it been possible to bypass infected natural sources to any significant degree by releasing clean prey in the peregrines' vicinity. The problem is that the falcons spend six months every year outside Sweden, when their diet cannot be controlled.

Threatened: the Peregrine falcon (Photo: Peter Lindberg)

Mr Lindberg is convinced that stable pesticides have done the birds more harm than any other single factor. Studies of the mercury content of Peregrine feathers have shown that until 1940, the mean mercury value was 2500 ng/g. In the 1940s an organic mercury preparation, alkyl-mercury, began to be used for dressing seeds to protect them against fungi. By the 1950s the falcons' mercury values had increased by ten and twenty times, reaching a peak just before the use of mercury was banned in 1966. Since then the mercury values have decreased, but in the early 1970s they were still three eight times as high as the pre-1940 mean. Not only is mercury harmful to grown birds, which become paralysed when poisoned, lose their balance and finally die, but it also reduces the chances that eggs will hatch—Peregrine eggs with a mercury content as low as 450 ng/g (dry weight) have been found unhatched.

Chlorinated hydrocarbons have also helped to reduce the falcon colony. Many of these substances are stable and are stored in the birds' fat, only to be metabolised in winter as their fat reserves are used. DDT has been widely used to protect young plants in Swedish forests until it was totally banned this year. In its broken-down form of DDE, DDT is thought to thin the eggshells, which can then crack and break more easily than normal eggs. Eggs examined in Sweden have shown that the mean thickness of the shell fell by 11.4% to 0.31 mm between 1946 and 1974.

In addition, PCB, which has been used in industry in Sweden since the 1920s, is known to disturb the body's enzyme and hormone balance, and researchers suspect it can reduce the falcon's ability to breed. There are no thorough figures on the PCB content of Swedish birds because so few have been found dead, but one suspected chain of contamination suggests that the PCB in paint used on boat hulls dissolves in the water to be ingested by sea birds later preyed on by falcons.

Since the Peregrine project began, all 568 nesting places known to have been used by the falcon in this century have been registered, and most visited. Wherever volunteers have found a pair, they have kept a 24-hour watch on the nest (sometimes using closed-circuit television cameras) over three-month periods from the laying of the first egg until the young have left the nest. Even though falconry and the hunting of the Peregrine are banned by law in Sweden, the nests may still be disturbed. Often the culprits are foreign.

The problems of saving the Peregrine are further complicated by its migration. Calculations show that 53% of young Swedish Peregrines and 40% of older ones have been shot outside Sweden. Other countries' hunting, falconry and pesticide laws are therefore of enormous importance in the species' decline: a decline which is being registered not only in this country but in the whole of Scandinavia, Europe, North America and the eastern part of the USSR. At a conference of the International Council for Bird Preservation in Vienna last October these problems were discussed, and resolutions were passed urging that activities harmful to the falcons be banned in each country. Those to which the Peregrine mainly migrates are Denmark, England, the Netherlands, France, Belgium and northern Spain. But—as the experience of the French has shown—even if hunting is made illegal, it often takes the hunters a few years to comply.

In a last-ditch effort to save the species in Sweden, Mr Lindberg has just begun a captive breeding programme. He has gathered three falcons from Scotland and one from Sweden, and is hoping that their offspring will build up a productive stock which he will then be able to re-introduce to the wild. No quick results can be expected, though. The birds must be hatched in captivity or captured when they are very young, and no Peregrine has been known to breed in captivity before the age of five. Judging by the rate at which the natural population has been declining, there will be a sad hiatus during which no Peregrine at all will exist in the wild in Sweden. ☐

Canada's Science Council reconsiders its role

from David Spurgeon, Ottawa

EVER since the creation of the Ministry of State for Science and Technology (MOSST) in 1971, the status of the older Science Council of Canada, formed in 1966, has been in some doubt. The council was formed as an advisory body to the federal government on science policy, but this was also seen as one of the ministry's roles. Now, through public pronouncements made over recent months on the redefinition of MOSST's role, the Science Council's position has become even less clear.

It appears that a good deal of soul-searching has been going on behind the scenes. Since a new chairman and a new executive director were appointed—both relatively young men with experience in industry—internal discussions have proceeded about the role and purpose of the Science Council, and a much more publicly visible, popular and practical orientation is envisaged.

The new executive director, John J. Shepherd, who is 46, was formerly chairman of the board of the high-technology firm Leigh Instruments of Ottawa. Josef Kates, the chairman of the council, is president of a Toronto-based firm of systems analysts. Shepherd believes that council has always tried to reach the public, mostly through its publications, but that the primary audience was other scientists, making its activities too much like "talking to ourselves".

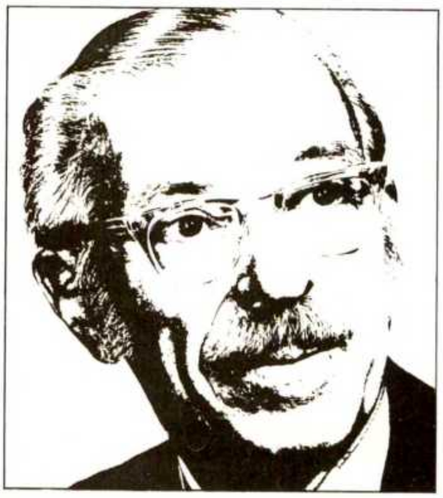
Now the appeal will be broadened—but with particular attention being paid to communication with industry. This month, the 20 Science Council members, 7 of whom are themselves from industry, will consider a paper that has been prepared to summarise the discussions already undertaken and present the views of the council's staff on the future role of the organisation. By early spring, it is hoped that agreement will have been reached on the council's purpose, some goals will have been accepted, and some decisions have been made on how to proceed to meet them.

This approach will contrast with more casual, *ad hoc* one of the past, and will, it is hoped, prevent endless debate on where the council is going. The kinds of proposals being made to the council in the paper that will be presented are these:

- The council should serve as an early-warning system to signal the need for R&D in specific areas important to the country. An international conference was sponsored by the council in Toronto in November, for example, on the subject of climatic change. Other possible topics for the future include the problem of fresh water supplies, and cold water problems for northern development.
- The council should define its role in international activities, particularly with respect to other countries' science councils.
- The council should take a view regarding some two or three important issues. One is the matter of basic research, and a second the question of technological sovereignty, that is, control over the technology the country acquires and the ability to exploit it nationally. Another is the concept of what the Council calls a Conserver Society, implicit in which are recycling and growth limitation. On the question of whether it can be sold to industrialists on the board, Mr Shepherd says that the Council's approach is that it would involve a lot of industrial opportunity. "It is not a negative, no-growth philosophy but a new opportunity".

One of the questions council members will doubtless ask themselves in considering this is whether they want to take the risk of committing themselves to such a specific policy. John Shepherd hopes they will, feeling that the council has nothing to fear from a re-structured and stronger MOSST. In fact he feels that the stronger MOSST can become, the bigger the public role of the council will be, because independent, though constructive, criticism will be needed all the more. But he sees the council as faced with a challenge in reaching the public: "There is a widespread feeling that R&D is not paying off. We've got to show very clearly how science is related to jobs". □

Equal time for nonsense



THOMAS H. JUKES

SEATED in a meeting of the Academic Senate at Berkeley, I was listening to the annual report of the Committee on Academic Freedom. It was one of those occasions when a printed copy is distributed prior to being read from the platform. The report contained the ringing words ". . . in areas of intellectual, scientific, artistic endeavour it is absolutely essential to protect and, indeed, to foster the odd-ball, the deviant, the genius, that blockhead who keeps insisting against all his colleagues that the earth is round and that it circles around the sun." With magnificent courage and perfect hindsight, I thought, we rally round Galileo to thunder defiance at the Inquisitors of 340 years ago, whose muddy vesture of decay keeps them from hearing us.

But what about the genius who insists that the Earth is *flat*? Especially the one who teaches this to university students. Let us remember that for every Galileo there were hundreds of odd-balls who kept proclaiming that the Moon was made of green cheese, or that the fault, dear Brutus, was not in ourselves, but in our stars, that we are underlings! Do we fight courageously, implacably, in the name of academic freedom, or the wider cause of freedom of speech and the press, to protect and foster soothsayers and alchemists? Should the Department of Astronomy appoint a Professor of Astrology because, as the resolution went on to say, "the intellectual deviant is one of our most precious resources?"

Don't overlook the fact that opponents of the scientific "establishment" are well aware of public sympathy for the underdog and of how easy it is to get equal time in the media. Some months ago I was asked to talk on television about nutrition. When I arrived at the studio, I found that the programme was to be spiced up by having me debate a "health food" salesman who had copped the spot by demanding "equal time". He regaled the audience with an account of the difference between natural and synthetic ascorbic acids. The natural variety, said he, bent a laser beam of light to the right, but synthetic ascorbic acid turned it to the left. Of course, I told the audience that it ain't so, but it was another case of disagreement between experts, and the television station loved it. Nothing like a one-against-one controversy to please listeners and boost the ratings.

So ended my effort on this occasion to bring nutrition from the ivory tower to the public ear. I departed, still dedicated to free speech, but well aware that those who own and control television stations and newspapers decide who gets it. And, right now, the odd-balls are doing very nicely. Acupuncture today, moxibustion tomorrow. Horoscopes were cast in a *Nature* article in April last year. We are approaching a state of affairs in which reactionary deviants who insist that the Earth circles round the Sun may indeed need protection.

Perhaps the Committee on Academic Freedom was right without really knowing why.

correspondence

Missing academicians

Sir,—The collective letter of 72 members of the USSR Academy of Sciences condemning the activity and stands of Andrei Sakharov (*Izvestia*, October 26) is worth analysis. According to *World of Learning* (1974–75 edition), the academy has 236 full and 445 corresponding members, that is, 671 in total. This means that only about 10% of the academy's members put their signatures on the anti-Sakharov statement.

The list of signatures does not contain the majority of the most famous academicians—physicists, who no doubt know better than others Sakharov's scientific and moral qualities. Among those physicists who did not sign the statement were P. L. Kapitsa, Bogolyubov, Migdal, Lifshitz, Zeldovich, Pontecorvo, Leontovich, Ginzburg, Budker, Belyaev, Frank, Cherenkov, Kadomtsev, Khariton, Kikoin, Linnik, Sagdeev and Vernov.

Similarly, we could not find among the 72 academicians the signatures of such leading Soviet mathematicians as Kolmogorov, P. S. Alexandrov, A. D. Alexandrov, Petrovskij, Pontryagin, Sobolev, Vinogradov, Kantorovich, Gelfand and Shafarevich.

In addition, the statement was not signed by T. Lysenko and M. Sholokhov. All told, the anti-Sakharov statement is much more a testimony concerning the real situation and moods in the Soviet Academy of Sciences than a simple condemnation of Andrei Sakharov

F. Janouch

Research Institute for Physics,
Stockholm

Crisis in Italian universities

Sir,—The article by Gillian Boucher entitled "Continuing crisis in Italian Universities" (November 20, p190) should, I feel, be completed by a few comments to give a fair representation of the situation. The comments which follow are based mainly on the situation in most of the Departments of Physics in Italy (which I do know from personal experience) but can apply with minor changes to almost all the faculties, the problems of Law, Engineering and Medical Sciences are similar in some respects, but different in others.

The central point of the article, namely that the Italian universities are in a state of agony, is correct, and this may, after all, be the only fact of general interest to your readers. It is also true that, due to a set of demagogic laws, any student can rather easily get his final degree; this is not stated explicitly in the article, but can perhaps be inferred.

What is omitted altogether is the fact that the demagogic laws mentioned above achieved the intended aim of introducing into practically permanent staff positions a large number of people with no scientific qualification but disposed to aggressive political activity (95% or more of them are leftists). This is the main reason why, in spite of the present political turbulence, the invasion of departments is much less frequent than it used to be a few years ago. those who invaded or stimulated the invasions now have permanent positions inside.

This process, in addition to depressing the average scientific level in many departments to unbelievably low values, has filled each department with large numbers of "teachers" or post-doctoral "research workers". They teach or do research only in Marxism, or, to be generous, in some cases, in Science and Society To give an example, the number of staff members with an office in the Department of Physics in Genoa is ninety-five; the total number of students in Physics, covering all the four years of the curriculum, is approximately the same. Some teachers do not have any students at their courses.

This overcrowding of "democratic" but ignorant staff members in the departments has three important consequences:

- those people who have obtained their positions fighting against "meritocracy" continue to dedicate all their activity to similar issues;
- they reproduce themselves;
- they occupy, for ever, a large number of positions.

In these conditions no brilliant young man has a chance of finding even a temporary position in the university.

These remarks show, I hope, that the main problems of the Italian universities are not of money (which is, however, badly administered), nor the fact that the full professors try to conserve their privileges (as one of the privileged I would like very much to know from your correspondent what my privileges are). The main problems are the demagogic and antimeritocratic edicts which were strongly supported for many years by the *Corriere della Sera* (to which Gillian Boucher refers). It is a well known tactic of disruption to destroy something first and then to complain about how badly it works.

Giacomo Morpurgo

Department of Physics,
University of Genoa, Italy

Kennedy's deed

Sir,—You include a comment (November 6, p5) entitled "Kennedy's good deed" regarding the U.S. Senate vote to ban the use of diethylstiboestrol as cattle feed supplement. A number of controversial matters are introduced into this short article, but I wish to protest the attribution of purely political motives to Senator Kennedy's sponsorship of this bill. Those of us who know of Senator Kennedy's past efforts on behalf of cancer research in this country must consider these remarks both unfair and uncalled for. One would hope for much higher standards from *Nature*.

George P. Studzinski

New Jersey Medical School,
Newark, New Jersey

Stockport to Darlington?

Sir,—I have just seen your issue of September 25, in which you quote from an 1875 *Nature* in *A hundred years ago* The extract deals with the opening of the first railway, but refers to the line "between Stockport and Darlington". Is this a 100-year-old misprint, or one perpetrated as recently as September 1975?

I am reliably informed that it is not even today possible to go from Stockport to Darlington direct: the trip necessitates two changes of train and takes 3–4 hours. The distance between Stockton and Darlington, which gave Stephenson's railway its correct name, is 30 miles or so.

B. Jove

Hove, UK

Unfortunately the error was made in 1875, and has been faithfully reproduced! —Ed.

Box and Cox

Sir,—Cox (August 14, p524) writing on Box! You're having us on.

R. S. Bray

Medical Research Council,
The Gambia

news and views

Low grade fuel: burning problems solved?

from Allan N. Hayhurst

In a recent article (*Nature*, **257**, 367; 1975) Lloyd and Weinberg of Imperial College, London, point to the immense waste of energy by the discharge of low grade fuels to the atmosphere. They quote as common examples the direct venting to the air of upcast gases from coal mines, of methane from sewage or wastes fermenting in air and also of the wide variety of exhaust gases from a great range of industrial processes. It has been argued, for instance, that all the machinery of the British coal industry could be powered by burning the methane present in the ventilation air from mines. Also, large industrial flares appear to everyone as a common and puzzling waste of good fuel; and of course, all this is to say nothing of the pollution associated with some of these fairly frequent practices. Lloyd and Weinberg also feel strongly on the point that until recently many combustion devices have been very simple because often there has been little incentive to engineer or design more efficient plant. The potential savings are clear, for they maintain that an improvement in the use of energy from combustion systems of only 12.5% could, at this moment, replace all other present sources of power, including nuclear.

Another aspect of the picture is that it seems certain that in the future prime hydrocarbon fuels will increasingly have to go to the chemical industry for processing, because in terms of world resources they are or will become too precious to burn. There will be an increasing need to burn only low grade fuels, which must include domestic and industrial waste. One great difficulty here is that methane, say, will not burn if diluted with air to more than twenty times its own volume. This is the problem to which Lloyd and Weinberg address themselves, namely that of devising a way of burning very lean gas mixtures, which are normally well beyond the limits of flammability.

Their interesting contribution is to construct a burner with a cylindrical geometry, which in cross section looks like a swiss roll. The mixture of air and fuel spirals in an elongated channel from the periphery towards a combustion chamber on the axis of the arrangement, while the products of combustion emerge from the middle in the space between adjacent layers of entering fuel and air. The purpose of such a configuration is to provide a large recirculation of heat, so that an appreciable fraction of the energy released by combustion is transferred back to the entering feed gases. By minimising in this way the loss of energy from the burner at the centre, the temperature there is forced to rise to levels much higher than would be obtained without heat recirculation. This 'amplification' of the temperature by concentrating heat in the combustion chamber facilitates the stable burning of very lean mixtures of fuel. For instance, in a small scale investigation at Imperial College, a mixture of 1% of methane and air was burnt to give a temperature of 1,400 K, when normally the flammability limit is 5.3% of methane in air. Further improvements seem possible by increasing the extent to which heat is recycled and also by providing extra lagging for the entire arrangement. It should be noted that nothing particularly special has been done to the burner, which can be an ordinary combustion chamber or even a fluidised bed. Certainly one effect of these findings is to extend the idea of a fuel to almost anything that can generate heat, no matter how small in amount compared with that from conventional high grade fuels. Lloyd and Weinberg thus envisage this as enabling us to extract the energy from those unburnable waste gases and also to minimise some of our air pollution at the same time. In addition, they mention the possibility of humidifying, heating and sterilising the air in hospitals by removing it to burn small quantities of hydrogen in it.

One important consideration of these devices is the efficiency with which energy can be taken from them and converted into, for example, mechanical work in an engine. On this question of thermodynamic efficiency the authors conclude, at first sight possibly surprisingly, that the conversion of the heat of combustion into other forms of energy can be achieved with a greater efficiency if the fuel is first diluted with excess air and then burnt in this new way. In fact, heat recirculation appears to increase the potential (or Carnot) efficiency from 60 to around 77% for a flame temperature of 1,500 K and herein lies much of the attractiveness of the device. In their extensive consideration of all the possible uses of the swiss roll burner, mention is also made of heating some other fluid, which, for example, undergoes an endothermic chemical reaction at high temperatures. Clearly they have in mind the production of a higher grade fuel from a low one, with the water–gas reaction or reforming as possibilities.

These burners based on large heat recirculation have been described before (*Nature*, **233**, 239; 1971 and **251**, 47; 1974) by Professor Weinberg. A very strong case has now been presented for quickly taking their development a stage further, by for example, building larger versions to discover the problems of operating on a practical scale. For example, will start up be a problem after what is in effect a burner in a heat exchanger, has been scaled up? Also, just how economic will they be?

So far only gaseous fuels have been investigated in these laboratory experiments and clearly extension to solid and liquid ones is worth considering. Two solid fuels—coal and refuse—spring immediately to mind and having established a certain degree of feasibility with methane, it is important to study various possibilities for recycling heat in these cases. In addition, the manufacturers of conventional combustion equipment, whether it be of large industrial furnaces, small scale domestic burners, internal combustion engines, rocket motors or jet engines, will have to look at heat recirculation to see how some or much more of it can enable them both to burn lower grade fuels or increase the efficiency with which energy is used. One imagines that there might well be considerable opportunities for improving the performance of existing plant. Pollution will always be an important factor whenever fuels are burnt, and here there are real possibilities of reducing the amounts of carbon monoxide, unburnt hydrocarbons and nitric oxides discharged to the atmosphere. One problem with building up very large temperatures in a combustion chamber is the perennial one that the containing walls must neither melt nor oxidise. Even so, this new approach to combustion engineering, backed by some very telling work in the laboratory, offers immense potential to make a better use of scarce resources in a remarkably varied set of circumstances and conditions. ☐

Control of enzyme synthesis by steroid hormones

from Robert Shields

ALTHOUGH it is now clear that the basic control of gene expression in prokaryotes is at the level of gene transcription it is still a cliché to say that control in higher organisms is "less well understood". One mammalian system that has received a great deal of attention (especially in the laboratory of the late Gordon Tomkins) is the control of tyrosine amino transferase (TAT) synthesis by steroid hormones.

When glucocorticoids are added to a line of rat hepatoma cells (HTC cells) the rate of *de novo* synthesis of TAT increases. This increase presumably occurs through increases in the level of TAT mRNAs, as inhibitors of RNA synthesis such as actinomycin D (Act D) will prevent induction and steroids do not regulate TAT synthesis at the translational level (Scott *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2937; 1972). If the hormone is washed out of the cultures the level of the enzyme falls (termed deinduction), but surprisingly, if Act D is added at the same time, the enzyme levels do not fall and may even increase (superinduction). On the basis of these observations Tomkins and his collaborators suggested that TAT synthesis was governed by two genes, one being the structural gene for TAT mRNA, and the other coding for a labile post-transcriptional repressor which destroys TAT mRNA (Tomkins *et al.*, *Science*, **166**, 1474; 1969).

In the original model steroids were envisaged acting post transcriptionally by interfering with the action of the repressor and leading to its destruction, thus allowing TAT mRNA to accumulate. When the inducing steroid is removed, the model predicts that repressor levels will rise and TAT mRNA will be destroyed. The maintenance of TAT levels in the presence of Act D (even after steroid removal) was explained by the drug inhibiting repressor synthesis, resulting in the labile repressor disappearing and TAT synthesis continuing unimpeded. Unfortunately it has not yet proved possible to quantify TAT mRNA directly, so its levels are inferred indirectly from the rates of TAT synthesis (Steinberg *et al.*; *Proc. natn. Acad. Sci. U S A.*, **72**, 2007; 1975).

The original evidence for post-transcriptional regulation depended heavily on the maintenance of TAT levels by Act·D after inducer removal. Act D might stabilise TAT mRNA (as required by the model), or more prosaically, it might interfere with TAT degradation and so maintain high levels of the enzyme. This last point has been the source of some controversy. When HTC cells are growing rapidly the half life of TAT degradation is about 3 h and is unaffected by Act D (Tomkins *et al.*, *Nature new Biol*, **239**, 9; 1972). If the cells are not growing because of nutritional deprivation, however, this rate is doubled and is slowed by Act D (Auricchio *et al.*, *Nature*, **224**, 806; 1969). These results showed that the maintenance of TAT levels by Act D in growing cells was not due to inhibition of TAT degradation Exactly the opposite interpretation was reached using another hepatoma cell line grown in monolayer culture (Kenney *et al.*, *Nature new Biol.*, **246**, 208; 1973). These workers produced evidence to show that in their experimental conditions Act D maintains TAT levels by interfering with TAT degradation. The rates they observed, however, were equivalent to the enhanced rate seen in non-growing HTC cells. If the cells used by Kenney were not proliferating rapidly, the results from the two groups are then not necessarily incompatible, Act D only affecting the rapid rate of degradation seen in stationary cells.

If the levels of TAT remain constant in the presence of Act D, then it follows that the rate of enzyme synthesis must be balanced by its rate of degradation. This rate is estimated by incorporation of radioactive amino acids into immunoprecipitable enzyme during a short pulse of label. Once again the results from different groups are conflicting—Tomkins and his co-workers reporting that Act D hardly affected TAT synthetic rates (Tomkins *et al.*, *Nature new Biol.*, **239**, 9; 1972), while results from another group reported that Act D inhibited TAT synthesis (Kenney *et al. op cit.*). Again the discrepancies can be explained by a careful scrutiny of the experimental procedures. Tomkins and his group express their result as 'relative rate' of TAT synthesis, that is, incorporation of radioactivity into immunoprecipitable TAT as a proportion of the total incorporation into cell protein. Kenney's group expresses its results as radioactivity incorporated in TAT. A careful study of the rates of TAT synthesis at various times after inducer removal and after addition of Act D reconciles these observations (Steinberg *et al*, *Cell*, **5**, 29; 1975). Whichever way the results are expressed it is clear that Act D will stabilise TAT synthetic rate (and by inference TAT mRNA) against the rapid decline of synthetic rate seen after inducer removal. This stabilisation is more dramatic when expressed on a relative rather than an absolute basis. These results suggest that TAT mRNA is stabilised relative to other mRNAs by the action of Act D and is consistent with the idea of a post-transcriptional repressor governing TAT mRNA stability If this interpretation is correct other inhibitors of RNA synthesis should also stabilise TAT levels after the inducing steroid is removed. Several inhibitors have been tested with different results. One, MPB, will maintain TAT levels and has no effect on enzyme degradation suggesting that TAT mRNA is stabilised by the drug (Levinson *et al., J. biol. Chem.*, **246**, 6297; 1971). Other inhibitors such as cordycepin and camptothecin fail to maintain enzyme levels unless Act D is also present (Dethlefsen, *J. cell. Physiol.*, **86**, 155; 1975; Bushnel *et al., Biochem, biophys. Res. Commun.*, **56**, 815; 1974). Both cordycepin and camptothecin affect protein as well as RNA synthesis, however, and so a decline in enzyme levels could mean that the inhibitors are affecting protein synthesis rather more than they are stabilising mRNAs. It is not possible to resolve this unambiguously without a more direct assay of TAT mRNA levels Fortunately, not all the evidence for post-transcriptional regulation of TAT mRNA levels relies on inhibitors. When induced HTC cells are enucleated and the steroid removed, TAT levels remain high in the face of normal enzyme degradation (Ivarie *et al., J. cell. Physiol*, **85**, 357; 1975). This strongly suggests that a nuclear function is required for the inactivation of TAT mRNA. The results of the Act D experiment suggest that this may be transcription of the post-transcriptional repressor gene.

If the post-transcriptional repressor exists, then steroid may regulate TAT mRNA accumulation either by interfering with repressor synthesis and/or action and so altering the rate of destruction of TAT mRNA, or by acting directly on control elements governing mRNA production. The kinetics of changes in mRNA levels on induction and deinduction would be different in the two models. Using rates of TAT synthesis as an indirect measure of TAT mRNA levels it is possible to fit the data to either model; however the model whereby steroids govern mRNA production rather than destruction seems to be rather more plausible and requires more reasonable assumptions (Steinberg *et al*, *Proc. natn Acad. Sci. U.S.A.*, **72**, 2007; 1975). The results do not define the point at which steroids act on mRNA production, but the relatively long lag between nuclear localisation of the steroid and the appearance of functional TAT mRNA might suggest that steroids do not govern gene transcription but some later stages in the processing of TAT mRNA precursors.

The picture that emerges from the work of Tomkins and his group is that eukaryotes may regulate the synthesis of certain proteins by regulating the levels of their respective mRNAs. This can be done not only by altering their rates of production but also by affecting the rates of destruction of these mRNAs. There is now abundant (admittedly indirect) evidence to suggest that many diverse cell types also exhibit a similar type of regulation (Tomkins *et al., Nature new Biol.*, **239**, 9; 1972). □

Proteins in and out of membranes

from a Correspondent

THE current model of a biological membrane is quite complex. The lipid and protein are seen to be organised as an intimate mosaic in which some of the protein is loosely attached to the surface of the phospholipid bilayer, some partly buried in the hydrophobic core of the bilayer and some spanning the bilayer Yet this model, which has gained general acceptance over the past decade, was preceded by a much simpler one, in which all the protein was seen to reside on the polar surfaces of the lipid bilayer, and which, for nearly thirty years, stood without being seriously challenged. When this simple model was replaced, it was not because the localisation of a protein within the lipid bilayer had been directly demonstrated but because it became difficult to reconcile the model with such overall physical properties of membranes as their optical rotatory dispersion spectra or such dynamic properties as active transport. This situation reflected the lack of reliable methods of determining the localisation of membrane proteins.

The elegant experiments of Henderson and Unwin (*Nature*, **257**, 28; 1975) on the photon-driven proton pump in the membrane of *Halobacterium halobium* have now provided direct and unequivocal evidence of a protein spanning the hydrophobic core of a membrane. Most workers in this field will, however, read this work with admiration but then return, to their own membranes the proteins of which cannot be induced, either individually or collectively, to form the regular lattice needed for resolution by the method used by Henderson and Unwin It is therefore appropriate that two papers should have appeared in the past few weeks that highlight the strengths, the difficulties and the weaknesses of some of the classical methods and which are also the latest results of two series of experiments that have led to the construction of a model for the membrane localisation of the glycoprotein, glycophorin. Glycophorin is a heavily glycosylated protein that can be isolated from human erythrocyte membrane It carries several biological activities including the MN-blood group activity. Tomita and Marchesi (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2964; 1975) have now published the complete amino-acid sequence and the oligosaccharide attachment sites of the protein. It consists of three distinct domains, two polar ones at the N and C-terminal ends and a non-polar segment in the centre. The sixteen oligosaccharides are all linked to the N-terminal polar domain, seven to serine and eight to threonine hydroxyl groups. Marchesi and his coworkers have suggested that the non-polar segment spans the hydrophobic core of the erythrocyte membrane leaving the N-terminal domain exposed on the outer surface of the membrane and the C-terminal domain on the inner.

The first worker to suggest that the protein did span the membrane was Bretscher. He treated intact red cells and ghosts with the vectorial probe, formyl-^{35}S-methionyl sulphone methyl phosphate (FMMP) This probe will not penetrate the membrane of an intact cell and thus labels only the protein exposed on the outer surface. Bretscher found that the glycophorin isolated from the ghosts was more heavily labelled than that from the intact cells and assumed that the ghost membrane was more permeable to the probe and that exposed protein on the inner surface had been labelled. His latest paper (*J. molec. Biol.*, **99**, 831; 1975) identifying the internally labelled fragments with those derived from the C-terminal end by the sequence workers greatly strengthens his assumption and the model.

The value of both sequence work and vectorial probe experiments is demonstrated by the result, and the difficulties, especially those found in subjecting heavily glycosylated proteins to standard analytical techniques, have been well described by both authors The papers also illustrate at least one pitfall. Marchesi's group had presented further evidence for the internally exposed domain in the lactoperoxidase labelling pattern obtained for glycophorin. It resembled the FMMP pattern in that the protein was more heavily labelled in ghosts than in intact cells. Lactoperoxidase is said to label only tyrosine and histidine but both the complete sequence work and Bretscher's amino-acid analysis of the C-terminal fragments show that these residues are absent from this segment of the protein Clearly one must know the precise specificity of a probe and the permeability of the membrane to it if it is to be of any use.

The biological activities of glycophorin, such as blood group activity and influenza virus binding capacity, do not immediately indicate the role of this protein in the membrane and the above model does not help much in this respect. The intramembraneous segment may simply be an anchor or possibly provides a channel for ion transport, though this function is usually ascribed to the other major glycoprotein of the red cell membrane. The predominance of adjacent threonine and serine links to the carbohydrate is unusual and in this superficial way the N-terminal domain has affinities with the gastric mucus glycopeptides. □

Polarised light and insect vision

from our Insect Physiology Correspondent

MORE than a quarter of a century ago von Frisch announced to a sceptical world that honeybees could orientate themselves by the pattern of polarised light in the blue sky. It was soon found that ants could do the same; that even insect larvae with simple ocelli had the same ability; and that crustacea living in the dim illumination of the sea floor were exceptionally sensitive to changes in the plane of polarised light. For a time it was argued by some authors that polarised light was not perceived directly but only after reflection from a dark background; but it is now well established that that is not necessarily so. The plane of polarisation can be perceived directly by the compound eye, but the mechanism of such sensitivity has remained a matter of continuing controversy.

It has been established that this perception has nothing to do with the structure of the cuticular cornea, nor with the crystalline lens; the analyser is clearly in the rhabdomeres, the retinal receptors. What has remained uncertain is whether it is the geometry of folding in the membrane of the rhabdomere that is responsible for a 'form dichroism', or whether, as in Polaroid, the molecules of the receptor pigment are themselves anisotropic or are so oriented as to provide an 'intrinsic dichroism'.

A recent paper by A. W. Snyder and S. B Laughlin (*J. comp. Physiol.*, **100**, 101; 1975) goes far to clarify this problem As with so many controversies both parties share the truth: the relative importance of form dichroism and intrinsic dichroism varies in different eyes. The eyes of vertebrates are not at all sensitive to changes in the plane of polarisation; but when the outer segment disks of the rods in the vertebrate retina are observed from the side, it is clear that they contain absorbing dipoles which are oriented at random in the plane of the membrane. The same applies to the microvillus membrane of the separated rhabdomeres of flies. On the other hand, in the fused rhabdoms of crustacea the dipoles are highly aligned along the axis of the microvillus membrane. It may well be that the rhodopsin molecule is asymmetric in crustacea and spherical in the fly microvilli.

The authors show that the amount of light absorbed by a photoreceptor depends on the orientation of the absorbing dipoles within its membranes: there is an optimum orientation for the photoreceptor to have a maximum absorption of unpolarised light, and this optimum varies with the organisation of the photoreceptor. In the vertebrate outer segment, maximum sensitivity is given by a random orientation in the plane of the membrane. In the unfused rhabdoms of flies the optimum sensitivity is again given by a random orientation of dipoles in the plane of the microvillus—and this gives a low dichroic ratio of about 1.7 to 1. In the fused rhabdoms of crustacea and of worker honeybees the dipoles must be highly aligned with the microvillus axis to provide the rhabdom with maximum absorption of unpolarised light; and this may yield a dichroism as high as 20 to 1.

Thus it would seem that the dichroism of the receptor membrane is merely a consequence, or by-product, of an adaptation which will provide the photoreceptors with optimum sensitivity to unpolarised light by maximising the effectiveness of every available rhodopsin molecule. The honeybee has utilised the bonus of polarisation sensitivity for practical purposes, in the orientation of its flights when the Sun is excluded from the field of view.

Inverse alpha decay

from P. E. Hodgson

THE spontaneous emission of alpha particles from radioactive nuclei has been studied since the earliest days of nuclear physics, and indeed Rutherford deduced the existence of the nucleus itself from the results of his experiments on the scattering by thin metallic foils of alpha particles emitted from natural radioactive sources.

It was soon found that the half-lives of radioactive nuclei, the times in which the intensity of alpha particles from a particular source drops to half its initial value, vary over a wide range from 0.3 μs to about 10^{17} yr. This remarkable range was explained quantum-mechanically by Gamow as due to the sensitivity of the rate of tunnelling through the nuclear potential barrier, of the wavefunction representing the alpha particle to the width of the barrier. With a reasonable model for the potential the experimental range of half-lives could be reproduced, and this was one of the first successful applications of quantum mechanics to the nucleus.

In subsequent years the theory of alpha decay has been developed in detail, and it has been found possible to express the probability of alpha emission as the product of the probability of formation of an alpha particle on the nuclear surface and the probability that it will penetrate the nuclear potential barrier. This enabled the relative values of a large number of alpha decay probabilities (related to the half-lives) to be calculated with good accuracy, but it was not possible to reproduce the absolute values because of the complexity of the nuclear structure calculation needed to find the alpha formation probabilities and the extreme sensitivity of the penetration probability to the details of the nuclear potential.

Nuclear structure calculations are easier to carry out for light nuclei because they have fewer nucleons, and there are several excited states of light nuclei that can decay by alpha emission, and this provides more favourable conditions for testing theories of alpha decay. Detailed calculations of the alpha decay of some excited states of ^{20}Ne by Arima and Yoshida (*Phys Lett.*, **40B**, 15; 1974) successfully gave not only the relative but also the absolute alpha decay rates in good agreement with the experimental data.

Another interesting way of testing theories of alpha decay is by studying the inverse process of alpha capture, and this has been made possible by recent studies of alpha transfer reaction (*Nature*, **252**, 270; 1974; **255**, 373; 1975). The main difficulty is that the cross sections of alpha-transfer reactions fall rapidly for heavier nuclei, but this has now been overcome by a group working at Rochester (De Vries *et al.*, *Phys Rev. Lett.*, **35**, 835; 1975).

They chose the (^{16}O, ^{12}C) reaction as it generally has a higher cross section than other alpha-transfer reactions, and measured differential cross sections of the ^{208}Pb(^{16}O, ^{12}C)^{212}Po reaction at 93 MeV to the ground and 0.727 MeV 2^+ states of ^{212}Po. These cross sections have the bell-shape characteristic of peripheral transfer reactions, and they were well fitted by finite range distorted wave calculations taking account of the nuclear recoil. The transferred alpha particle was treated as a single particle and no account was taken of its internal structure; this is called the cluster approximation. This analysis gave a ratio of spectroscopic amplitudes for the two reactions of S (0.727 MeV 2^+)/S (g.s.)=0.64±0.13. Only the ratio could be obtained as the cluster approximation introduces unknown uncertainties in the absolute value of the calculated cross section.

The nucleus ^{212}Po also decays radioactively, and the analysis of the half-lives for alpha emission gives comparable spectroscopic information. Several different potentials were used to calculate the barrier penetration probabilities, and although the absolute values differed considerably they gave a consistent value of the ratio of spectroscopic amplitudes for the same two processes of 0.61±0.24.

Although they are still subject to considerable uncertainties, these two values of the ratio of the spectroscopic amplitudes from alpha transfer and alpha decay are certainly consistent, and provide good overall confirmation of the two theories used to obtain them. In particular, the agreement supports the interpretation of the alpha-transfer reaction as a single one-step process that can be treated by the cluster approximation. □

Aspirin's target in anti-inflammatory action

from R. J. Flower

THE multi-enzyme complex which synthesises prostaglandins (PGs) ("PG synthetase", "fatty acid cyclo-oxygenase") is bound firmly to the "microsomal" membranes of cells, a fact which has naturally proved very frustrating both to enzymologists and pharmacologists who would like to study pure preparations of this unique dioxygenase. A definite advance was made last year when three Japanese workers (Miyamoto, Yamamoto and Hayaishi, *Proc. natn. Acad. Sci. U S.A* , **71,** 3645; 1974) succeeded in solubilising the enzyme and resolving it into separate "oxygenase" (catalysing the transformation of endoperoxide intermediates) and "isomerase" (catalysing the transformation of endoperoxides to PGE_2) components.

Now another step forward has been made by a group at the Washington University School of Medicine who have further characterised this enzyme and reported that the catalytic unit is a protein of molecular weight 85,000. To accomplish this, Roth, Stanford and Majerus (*Proc. natn. Acad. Sci. U.S.A.*, **72,** 3073; 1975) used an ingenious adaptation of the finding that aspirin (acetylsalicylic acid) and its congeners inhibit PG synthetase (Vane, *Nature new Biol.*, **231,** 232; 1971; Smith and Willis, *ibid*, 235; Ferreira *et al.*, *ibid*, 237). Although the detailed mechanism of aspirin inhibition of the enzyme was unknown, the Washington University group used as a working hypothesis the known ability of the drug to acetylate proteins. After synthesising aspirin with a tritium label on the labile acetyl group, they incubated the drug with crude PG synthetase preparations derived from human platelets, and sheep and ox seminal vesicles. After incubation the enzyme was solubilised in sodium dodecyl sulphate and subjected to polyacrylamide gel eletrophoresis. The bulk of the radioactivity in each of the tissue digests was found to migrate as a protein of molecular weight 85,000. No incorporation of radioactivity was found when the aspirin was labelled in the aromatic ring. Evidence that PG synthetase (and not other microsomal proteins) was specifically acetylated came from the good correlation between the low concentrations of aspirin required for acetylation and enzyme inhibition and the rapid time taken for both reactions. The authors' claim was further substantiated when it was found that protein acetylation was blocked by the fatty acid substrates of the enzyme (arachidonic and dihomo-γ-linolenic acid) as well as by another potent PG synthetase inhibitor, indomethacin. Aspirin is thought to block the initial dioxygenase reaction and it would thus seem that the catalytic unit (or subunit) of the dioxygenase enzyme has a molecular weight of about 85,000, and that acetylation of the active site is the mechanism of aspirin inhibition—although as the authors are careful to point out, aspirin binding at a distant "allosteric" site could also prevent arachidonic acid binding at the true catalytic site.

How will this finding increase our understanding of PG synthetase? As well as contributing an important piece of information about the enzyme it suggests many other fascinating experiments: the acetylated protein could itself be digested and the labelled amino acid identified. This would be helpful in building up a picture of the enzyme's catalytic centre, and ultimately aiding the design of novel anti-inflammatory agents Pharmacologists will undoubtedly want to know whether any other "aspirin-like" drugs act in an analogous fashion, and perhaps the specific labelling of the enzyme with aspirin will help solve the vexed question—in which membrane fraction is PG synthetase actually located?

Because of the great diversity of structures amongst the aspirin-like drugs (see Flower, *Pharmac. Rev.*, **26,** 33; 1974) it would seem unlikely that they could all work by way of the mechanism which Roth and his colleagues propose. One obvious exception is salicylic acid itself which lacks acetylating capacity, and which *in vivo* (though not *in vitro*) is equally effective in inhibiting prostaglandin synthesis (Hamberg, *Biochem. biophys. Res. Commun* , **49,** 720; 1972), although there are differences in its latency of action. Coming as it does, however, in the midst of so many new developments in the PG area, this finding by the Washington University group could prove to be a timely stimulus to further research on the enzyme complex itself.

Christmas trees

from Peter D. Moore

As yuletide approaches, a botanist's mind inevitably turns to thoughts of the gymnosperms and their symbolic significance to man. From early prehistoric times certain members of this group, including that Christmas favourite, the spruce *Picea abies*, seem to have had their fortunes closely linked with the activity of man. For example, Markgraf (*Nature*, **228,** 249; 1970) has suggested, on the evidence of numerous radiocarbon dated pollen diagrams, that the spread of spruce in Switzerland occurred at different times in different areas, but was frequently accompanied by evidence of human disturbance of the forest, such as weed and cereal pollen. She felt that prior to disturbance the spruce was unable to invade the stable forests of *Abies alba, Pinus cembra* and the mixed deciduous forests.

Moe (*Bot. Notiser*, **123,** 61; 1970) has also shown that the spread of spruce in Fennoscandia was slow. It arrived in eastern Finland around 3,000 BC, but had still not penetrated far into Sweden by 500 BC. But Tallantire (*Nature*, **236,** 64; 1972; *Norweg. J. Bot.*, **19,** 1; 1972) does not regard man as the primary cause of its western migration in Fennoscandia. He believes that the distribution of spruce is essentially climatically determined, the critical factors being high summer and low winter temperatures. The spread of spruce, he claims, was a stepwise process, each advance corresponding to a period of suitable climate. He is prepared to admit that once established in an area its subsequent spread and population expansion in the locality could be assisted by human clearance of virgin forest. Thus, from microclimatically and edaphically favourable nuclei, spruce could attain a wide-

A hundred years ago

Mr. Casella, the well-known scientific instrument-maker, has sent us a specimen of a compass which will be a great boon to the many who are ignorant of the difference between the magnetic and the geographical poles, and of the fact that an ordinary compass points to the former and not to the latter, the difference in this country at present being about 19°. The great advantage of Mr. Casella's "unmistakable true north compass," is that it points to the true or geographical north, being corrected for use in the United Kingdom, and capable of adaptation to any locality in any part of the world. It is a card compass of beautiful workmanship, swings with perfect ease, and by means of a black cone on a white ground, the merest tyro can read it. It is made in various sizes, and sold at various prices, and deserves to come into extensive use.

from *Nature*, **13**, 135; October 16, 1875

spread distribution within the disturbed forest.

A recently published pollen diagram from eastern Finland by Hicks (*Commentat. Biol.*, **80**, 1; 1975) is of considerable interest in this debate. It shows an expansion of *Picea* at about 3,000 BC yet evidence of agriculture does not occur until a level dated at about 300 years ago. This is consistent with historic records of the invasion of the area by Finnish farmers during the 17th century. Previously northern Finland had been occupied by herding and hunting Lapps. There is still the possibility that some forest management, such as burning, could have been practised, however; perhaps the pollen type *Melampyrum* in Hicks' diagrams are indicative of this.

In Britain we have been spared these problems because *Picea* has never reached these islands during the present interglacial. An equally enigmatic problem, however, is posed by the marked decline in pine, *Pinus sylvestris*, since 5,000 BC and particularly in the last 4,000 years. This has affected the species in Ireland (Pilcher, *New Phytol.*, **72**, 681; 1973) where Bronze Age peoples may have been involved in the clearance of pine forests. Pennington (CBA Rept. No. 11, 74; 1975) has described a similar process from the uplands of the Lake District, though there the process began in Neolithic times. Birks (*Phil. Trans. R. Soc. Lond.*, **B270**, 181; 1975) has found that most of the pine stumps in the blanket peats of Scotland predate 4,000 b.p., but here death was frequently found to be due to rising water tables, possibly a consequence of minor climatic fluctuations. In none of the cases examined was it possible to blame man for the death of the trees. In the southern Pennines, on the other hand, Tallis (*Nature*, **256**, 482; 1975) regards the death of at least some of the trees preserved as stumps in the peat to be the result of human forest clearance.

In the case of both spruce and pine it is difficult to unravel the interacting influences of climatic changes and human clearance. It is evident, however, that man's involvement with these needle-bearing conifers began long before the yule log became a component of his culture. □

Does the Earth's field reverse?

from Peter J. Smith

The magnetic properties of natural lodestone have been familiar for a long time; indeed, the ancient Greeks knew at least 2,000 years ago that lodestone would attract pieces of iron, and by the first century AD the Chinese had invented the compass in the form of a lodestone spoon rotating upon a smooth board. In recent years, however, it has become apparent that even quite common rocks may exhibit magnetic characteristics. The fact that some eruptive rocks possess a natural magnetism much larger than can be acquired in the present geomagnetic field was reported by Arnim (*Ann. Physik*, **5**, 384; 1800) and confirmed by Locke (*Trans. Amer. Phil. Soc.*, **9**, 283; 1846). Fournet (*Ann. Sci. Phys. Nat.*, **11**, 134; 1848) has suggested that such a phenomenon is possible because of the presence in the rocks of magnetite. Unfortunately, this idea does little to explain how rocks can acquire an ordered magnetism; and the role, if any, played by the Earth's magnetic field remains obscure.

The mystery has now deepened with the publication of an article by Broun (*Geol. Rep. R. Soc. Lond. Trans. Sect.*, 24; 1860), who concludes that the direction of magnetism in some rocks may be quite different from that of the geomagnetic field. The rocks in question come from Moocoonoomalley, a granite hill which rises some 800 feet above sea level near Trevandrum in India. Broun has taken several samples from the hill, having previously marked their positions with respect to the geographic coordinates, and has measured their effect on a freely suspended magnet at various orientations.

All the samples studied have determinate magnetic axes with north and south poles at opposing faces. And the magnetic axes are always aligned roughly geographic north-south, suggesting that the magnetism owes more to the effect of the Earth's magnetic field than to any natural divisions or 'lines of crystallisation' in the rocks themselves. But whereas in most of the samples the north magnetic poles were directed towards the true north as expected, in some the north poles pointed south. Broun concludes that in the latter cases "we cannot suppose that the magnetism of the small magnets has been due to the inducing action of the earth in their present position or since the rock mass became solid". There is a clear implication here that these rocks acquired a permanent magnetism before, or as, they cooled from their molten state, presumably under the influence of the Earth's magnetic field.

But could this possibly mean that at some time in the past the Earth's magnetic field pointed in the direction opposite to the present direction, giving rise somehow to rocks with 'reversed' magnetism? Broun is not so bold as to suggest any such thing him-

de Castro: first discoverer of reversed magnetism?

self; but his work inevitably brings to mind two previous articles which went largely unnoticed at the time of their publication. Some years ago, Humboldt (*J. Phys. Chim. et Hist. Nat.*, **2**, 314; 1794) reported that when he brought his compass near a large mass of very pure serpentine atop a granite hill in the Fichtel Mountains of Upper Franconia, the needle completely reversed its direction. His conclusion was that the magnetic axes in the serpentine are parallel to the Earth's field but 'reversed' in polarity. Unfortunately, measurements carried out on the same rock by others in 1816 indicated that the polarity reversal was less complete over the whole mass of serpentine than Humboldt had thought, raising some doubt as to the true picture. Seen in the light of the more detailed study of reversed magnetism by Broun, however, Humboldt's discovery must be taken more seriously than hitherto.

An even older example of a compass needle reversing its direction was presented by Castro (*Logbooks of João de Castro*, Portugal, 1538–1539). During an expedition to the coast of India, Castro landed on the Island of Chaul and, placing his compass on a boulder in order to measure the bearings of the island, found that the north end of the needle pointed south. Although he made sure that the rock was not lodestone, Castro failed to record whether or not the boulder was in its original position of formation. The direction of the magnetism in it therefore remains unknown. But magnetised the boulder certainly was; and so Castro must be credited at least with the discovery that rocks other than lodestone can be magnetic.

In summary, then, Castro discovered magnetic rocks, Humboldt discovered reversely magnetised rocks and Broun has demonstrated scientifically that reversed magnetism is genuine. Taken together these are exciting results which pose some fascinating problems for the future. In particular, is a reversal of the Earth's field polarity really conceivable? If, as some still believe, the Earth is less than 6,000 years old, such a reversal might pose several problems of timing, for it is hardly to be imagined that so radical a change in so fundamental a phenomenon as the Earth's magnetism would be rapid. On the other hand, if, as Phillips (*Life on Earth*: *Its Origin and Succession*, Macmillan, London, 1860) suggested earlier this year, the Earth is 96 million years old, this difficulty would recede. □

The Yellala (rapids) of the Congo River.

Two Trips to Gorilla Land and the Cataracts of the Congo. By Richard F. Burton. Two vols. (London: Sampson Low and Co., 1876.)

The chief interest of the second volume is connected with the Congo river, up which Burton journeyed as far as the Yellala, or rapids, which he calculates to be between 116 and 117 miles from the mouth, the total fall in that distance being 390 feet, of which 195 feet occurs between the Yellala and Boma, 64 miles. From the Great Rapids to the Vivi or lowest rapids, a distance of five miles, the fall is 100 feet. Some important facts are given as to the character of the Congo mouth and the changes which are constantly taking place, which must even yet be of value to chart constructors.

The author gives a minute and graphic description of the river and its many reaches between Boma and the rapids; the scenery on the banks is often quite Rhine-like in its character. The river itself Capt. Burton regards as one of the noblest in the world. With a valley area of 800,000 square miles, it has a yearly mean volume of 2,500,000 cubic feet per second, nearly four times that of the Mississippi, which has a very much larger drainage area.—(*Nature*, **13**, 123; October 16, 1875).

Second messengers in synaptic transmission

from a Correspondent

A satellite symposium to the Fifth International Meeting of the International Society of Neurochemistry entitled New First and Second Messengers in Nervous Tissues, was held at Brescia, Italy, on August 28–30, under the auspices of the Department of Pharmacology of the Brescia Medical School. It was organised by the Chairman, Dr Marco Trabucchi.

CYCLIC nucleotides that function as second messengers in synaptic transmission not only mediate the first messenger-elicited changes in ionic membrane mechanisms but also participate in the regulation of the expression of the metabolic code. They can either enhance template activity or increase RNA polymerase II activity. This important progress in our understanding of how metabolic responses are regulated trans-synaptically in postsynaptic cells was reported during the symposium.

D. M. Chuang *et al.* (National Institute for Mental Health, Saint Elizabeth's Hospital, Washington, DC) reported that in rat adrenal medulla during a trans-synaptic sustained stimulation of postsynaptic nicotinic receptors or by the injection of specific receptor agonists, the 3′,5′-cyclic adenosine monophosphate (cyclic AMP) content increases by about 10-fold. The onset of this increase is coupled with stimulus application, but its duration is regulated independently. They have shown that a complex self-regulatory mechanism involving protein kinase, an activator of cyclic nucleotide phosphodiesterase and a high K_m cyclic nucleotide phosphodiesterase regulates the extent and duration of the cyclic AMP accumulation elicited in chromaffin cells by sustained trans-synaptic stimulation of adenylate cyclase. A low molecular weight protein, stored in the membrane of chromaffin cells, can be released *in vitro* from membrane preparations of adrenal medulla following phosphorylation of membrane proteins by a cyclic AMP-dependent protein kinase. As described by Cheung (*J. biol. Chem.*, **246**, 2859; 1971), this low molecular weight protein activates cyclic nucleotide phosphodiesterase by lowering the K_m of the high K_m enzyme which possesses a high catalytic capacity. Hence, in chromaffin cells, by lowering the

K_m of the phosphodiesterase the trans-synaptically induced increase of cyclic AMP content can be terminated even in the presence of persisting trans-synaptic stimulation of adenylate cyclase. When the cyclic AMP content of chromaffin cells is enhanced for one hour or longer, the cytosol protein kinase—the receptor protein for cyclic AMP—is activated. This enzyme is formed of two subunits: a regulatory (R) and a catalytic (C) moiety; normally the inactive RC molecular configuration is the most abundant form of the enzyme. When cyclic AMP increases it binds to R and dissociates the RC complex. As a result, the protein kinase activity is enhanced because when C is dissociated from R, it can express its catalytic activity at a maximum. Following persistent trans-synaptic activation of chromaffin cells most of the protein kinase molecules in these cells are dissociated and the C form prevails. This increase of C subunits lasts for about 4 h; however, the duration in the decrease in the number of RC molecules lasts for about 15 h. A. Guidotti and collaborators (NIMH) have reported that the mechanism whereby the trans-synaptically induced increase in C subunits is terminated is not by the reassociation of C with R but by the translocation of C from cytosol to subcellular structures including the nucleus. Chuang and E. Costa (NIMH) have shown that coincident with this translocation there is an increase in the synthesis of nuclear poly(A)-RNA. This elevation of mRNA synthesis rate is followed by an increased synthesis of tyrosine-3-mono-oxygenase (TH), the rate limiting enzyme for catecholamine biosynthesis. Also the synthesis of other medullary protein is enhanced at this time.

D. Russell (University of Arizona) also reported that the cyclic AMP content of adrenal medulla increases following the trans-synaptic activation of nicotinic receptors. She has shown that this second messenger response triggers the activation of ornithine decarboxylase which, in turn, accelerates the synthesis of ribosomal RNA.

R. A. Jungman and E. G. Kramias (Northwestern University Medical School, Chicago) have suggested that during hormonal regulation the RNA polymerase II activity can be modified through a phosphorylation of the enzyme by the action of a nuclear cyclic AMP-independent protein kinase.

I. Hanbauer (National Heart and Lung Institute, Bethesda) reported that when rats are maintained for about 1 h in an environment with low O_2 tension, the TH activity of the carotid body increases after an interval of about 24 h following the stimulus. This increase in TH activity is also preceded by an activation of the cyclic AMP system. She reported that this chemical stimulus fails to change the TH in adrenal medulla or in superior cervical ganglia; moreover, she presented evidence that this TH activation does not require the participation of adrenergic or cholinergic mechanisms.

During this symposium, E. Giacobini (University of Connecticut, Storrs) and M. Otsuka (University of Tokyo) and others presented substantial experiments supporting the view that first messengers may include not only amino acids and their metabolites, but also polypeptides and adenosine triphosphate. The biochemical and functional implications of the participation in synaptic transmission of several molecular entities of transmitters were debated. It was also discussed whether the presence of such a pluralistic system of first messengers may require an updating of the Dale postulate that each neurone contains one and only one transmitter. □

Fighting erosion with soil conditioning

from A. J. Low

The third international meeting on Soil Conditioners was held at the University of Ghent in Belgium on September 8–12. The proceedings of the symposium (edited by M. De Boodt) will be published by the Faculty of Agricultural Sciences of the University of Ghent.

SOIL is a three phase system—a porous heterogenous mixture of solids (minerals and organic matter), liquid (water containing many solutes) and gases. The structure of a soil is determined to a large extent by the soil organic matter which holds together the mineral skeleton. Soil structure is developed by the action of the root systems of permanent covering vegetation, such as that of grasslands. But when grassland is cultivated, the organic matter which has formed is quickly broken down and there is a corresponding structural collapse. The natural soil 'cements' include polysaccharides and polyuronides, and soil conditioners are synthetic replacements for these.

The first soil conditioners were probably vinyl acetate—maleic acid and hydrolysed polyacrylonitrile. Mixed with soil at a suitable moisture content they can bring about a much more stable structure which can last for ten years or so. Marked increases in crop yield have occurred when structure has been the limiting yield factor, but soil conditioners have not generally been adopted on a large scale because of their high cost.

What may be an important development is the application of soil conditioners in arid regions such as North Africa where to prevent the spread of the desert northwards an attempt is being made to establish a belt of trees (the Barrage Vert). The desert soil is largely devoid of organic matter, unaggregated and subject to wind erosion. It can be stabilised by building aggregates with soil conditioners and thus providing the conditions for trees and other plants to become established after planting which in turn helps to reduce wind erosion.

Suwardjo and Lenvain (Bogor Soil Science Institute, Indonesia) described how re-afforestation was being carried out in the humid tropics on strongly eroded areas where the soil structure was weak, by stabilising the soil in and around the planting holes of young trees. Once the trees are established the surrounding area tends to become revegetated.

W. Flaig (West Germany) related work on the biochemistry of soil organic matter to work on soil conditioning. He concerned himself especially with high molecular weight naturally occurring organic polymers and briefly discussed the possibility of converting lignosulphonates and other waste products into soil conditioners. The soil conditioning effects of lignin derivatives, sewage sludge and other waste products were also reported by Vasiljev (USSR), M. B. Kirkham (USA) and Knottnerus (Netherlands).

Fundamental studies of soil conditioners as materials for changing soil physical properties were described by De Bisschop (Ghent University) and Koenigs (Wageningen University) particularly liquid distribution in porous media.

Soil conditioners have been used to stabilise the backfill of pipe drains (Dierickz and Gabriels, Ghent University), in rice culture (Keersebilck and Muljadi, Bogor) and in stabilising sugar beet seed beds where soils are liable to cap.

The symposium concluded with an analysis of the papers and the conclusions to be drawn by Dudal (FAO) who suggested that soil conditioning might now be ready for application on a larger scale than hitherto. In contrast to the first symposium more than 50% of the papers in the third dealt with the use of waste products as soil conditioners. This was undoubtedly due to economics. The synthetic polymers used as soil conditioners in the 1950s were even then very expensive and continue to be so.

The fourth symposium is to be held in Australia on August 16–23, 1976.

Christmas quiz

1. (*a*) Did Mendel have to cheat to discover the fundamental laws of genetics?
   (*b*) Why were his results not recognised for forty years?

2. Norbert Wiener, Julian Schwinger, James Watson and Murray Gell-Mann were all child prodigies. But which was the most prodigious?

3. James Sumner and John Cornforth both obtained Nobel prizes for chemistry, albeit 29 years apart. What else did they have in common?

4. A question for women's year: The female is often larger as well as more deadly than the male. In which species is the imbalance greatest?

5. In which film, made in 1944, was there a sub-plot of German agents trying to smuggle uranium out of America?

6. In which book:
   (*a*) was there a submarine powered by the same force as powers the Sun (written 1870);
   (*b*) did Japan launch a surprise aerial attack on the west coast of America (written 1908);
   (*c*) did scientists develop strains for biological warfare (and subsequently use them on China, the emergent world power) (written 1914);
   (*d*) did a group of 'conservationists' fight the profligate squandering of natural resources by big business (written 1952)?

7. Whose correspondence was found recently in the Old and New Worlds, and what was the subject discussed?

8. For they have sown the wind, and they shall reap the reward. When was this biblical warning renewed recently, and what human activity was said to be the cause?

9. Which animal with ecclesiastical connections may aid investigations of human demyelinating disease?

10. What was the first anorectic drug used in the treatment of obesity?

11. What effect is season of birth supposed to have on the incidence of schizophrenia?

12. When was the first purely scientific journal founded?

13. Who's got arms coming out of his head?

14. Who invented the linear motor and ran away from the Royal Institution?

15. Who began by counting from 1 to 10 in binary form, added on the atomic numbers of hydrogen, carbon, nitrogen, oxygen and phosphorus, then twice 4,000, and drew a cuboid biped 14 wavelengths high with a helix on its head? And who turned it upside down?

16. In spite of the demise of flower children, strings of beads are still in favour with one group. Who are they?

17. Who said:
    (*a*) Science is the cemetery of dead ideas;
    (*b*) Science is the topography of ignorance;
    (*c*) Science is nothing but perception;
    (*d*) Science is the great antidote to the poison of enthusiasm and superstition?

See page 556 for answers.

Miscellaneous intelligence

- "Contrary to the trends in all other countries in the European Economic Community the rate of movement into human consumption in Great Britain increased during the three previous seasons (and shows a provisional offtake for 1974/75 of 222 lb per head per annum)." So says the Potato Marketing Board in its latest annual report, meaning that although the British people had been eating more potatoes during the past few years, they now seem to be eating fewer. Does this indicate that they might be in a spud-deficient situation?

- Oh for the elegant style of yesteryear. Lecturing at Gresham College, London on October 26, 1681, John Flamsteed, first Astronomer Royal, warned: "Hee that has not a Competent understanding in Geometrie, a thorough knowledge of plaine & sphericall Trigonometrie, with the Severall Theoryes of the planets motions, hee that with these is not well acquainted with the Sphere it selfe, & its severall projections & knowes not readily the Constellations, ought not to pretend to the businesse in which all these are required . . . " For further enjoiement, even if you knowe not one end of a telescope from ye other, see ye full texte in *The Gresham Lectures of John Flamsteed,* edited by E. G. Forbes (Mansell, London, 1975).

- A recent joint meeting of the Society of Chemical Industry and the Society for Occupational Medicine heard the momentous news that dialogue between the professions of chemistry and medicine was difficult in that each tended to use its exclusive jargon.

- Elsevier has published yet another journal. *Mass Emergencies* is intended for everybody concerned with problems of emergencies and disasters. It is to be hoped that it soon tells them what to do about an avalanche of new journals.

- A new book from Oxford University Press has a chapter—Education: how to make the best use of your brain—which might have been the answer to many a prayer. Sadly for aspiring geniuses, it describes no tricks for improving the power of mind over matter. Keeping to the realms of the possible, it deals largely with the acquisition of human skills. (*Explaining the Brain* is by Ritchie Russell with A. J. Dewar.)

- Television talks for small planters have been delivered by the staff of the Mauritius Sugar Industry Research Institute, according to the institute's latest annual report. Bad luck tall planters.

Results of competition No. 3

Examples of momentous discoveries happened upon by chance proved disappointing, and no first prize has been awarded. Consolation prizes go to the authors of the stories printed below, although one contains a historical inaccuracy.

"And now, allow me to demonstrate my new, cheap, thin-wire, glass enclosed heater", said Edison, slowly closing the switch. The ammeter flicked over and the lab. was lit up by a flickering glow. "Of course this is just to show the principle", he added heartily. "The production model will have opaque glass walls and a heat efficiency better than 80%". D. R. Reed

It is said that Arne Tiselius developed cellulose column chromatography from an ancient practice of Swedish peasants. While on holiday in the forests of western Sweden he came across an old woodcutter who, to prepare lunch, broke the ends off a long loaf of bread, stood the truncated part upright in a jar, and poured into the top of it a crude distillate of pulped leaves; the woodcutter ate his crusts after dipping them into the liquid which eventually filtered into the jar, and gave his dog the rest of the loaf. Realising that he had witnessed an ingenious method of separating ethyl and methyl alcohols, Tiselius returned to his laboratory pondering on how to apply it to the purification of proteins. D. Rosen

It is reported that Gregor Mendel once made a courtesy visit to a neighbouring nunnery. A keen gardener himself, he was much impressed by the magnificent sweet pea beds leading to the main entrance. The gardener, a tall, handsome, young red-haired man (rare in those parts of Moravia), accepted Mendel's compliment as nothing less than his due.

The abbess showed Mendel around, and was particularly enthusiastic about the success of their orphanage and school. Mendel, however, was more taken with the surprisingly large number of tall, pretty, red-haired children attending. "Strange", he thought to himself . . .
F. H. Cooper (after Boccaccio)

"Get back here and put some clothes on!" shouted Mrs Archimedes.

By that time, however, her husband was halfway to the palace, and Mrs Archimedes was left standing ankle-deep in spilled bathwater. How could she possibly clean that mess? Suddenly she noticed that the hem of her gown was soaking up some of the spillover. With a shout of "Eureka!", she quickly tore up some old clothes, tied them to a stick, and invented the mop.
S. Gilbert

Competition No. 4

One of the most popular sections of *Nature* is 100 Years Ago. Readers are invited to submit 100 words culled from the pages of *Nature* 100 years hence. Closing date: February 1, 1976.

Photo by A. Friday

This vicious beast was caught by natives in deepest Zaïre on behalf of two British zoologists who risked disease and discomfort to secure a sample of its blood. In spite of its fearsome appearance, this West African otter shrew, here slightly larger than life, is stuffed and safely at rest in the Museum of Zoology at Cambridge (see *Nature*, **258**, 107; 1975).

Ghost lecturers

When Professor Richard Eakin's lectures declined in popularity and audiences dwindled, he hit upon a topping wheeze. To revitalise his course in elementary biology he would bring in guest lecturers of exceeding eminence—Mendel, Darwin, Pasteur and their ilk. Being a thorough worker, and having at his disposal the considerable resources of the University of California, Berkeley, he has been able to transmogrify himself into various scientists who long since passed into the annals of history.

Thus when it is time to discourse on blood, an Elizabethan gentleman steps forth, carrying a Latin treatise, a heart and a pitcher of tomato juice. He is William Harvey and the props serve to demonstrate his work on the circulation of the blood. Later during the course of thirty lectures (most of which are delivered by Professor Eakin playing himself) Harvey is followed by William Beaumont, the gruff nineteenth-century American army surgeon who pioneered the investigation of gastric juice and digestion. He holds forth with some relish about his investigations with his patient, and later guinea pig, Alexis St Martin, a fur trapper whose stomach was shot open and remained open through the wall of his abdomen even after his recovery.

Embryology is introduced by a frock coated Herr Professor Dr Hans Spemann, the only character in Eakin's repertoire whom he knew. Genetics is brought into the course by a jolly, cigar smoking Brother Gregor Mendel, complete with plastic sweet peas (always fresh and available). To add authenticity to his portrayal of Louis Pasteur, Eakin has taken lessons in the art of affecting a French accent. He is apparently acquiring the Queen's English to improve his interpretations of Harvey and Darwin.

After their local success, the guest lectures have now been published in a book with the alluring title of *Great Scientists Speak Again* (by Richard M. Eakin, University of California Press, Berkeley, Los Angeles, London, 1975, £4.50). Other biologists eager to give the best attended lectures are invited to get out their grease paint, wigs and pitchers of tomato juice. M.L.

Ode to an eddy

The mighty stream Agulhas
  Wends its wand'ring way
Down the coast of Afrik
  And on past Durban Bay[1].

Twas there we found The Eddy[2],
  Majestic and serene,
Cyclonically rotating:
  The best we've ever seen.
Its heart was cool yet shallow—
  Its motion slow and steady—
Its structure well-defined—
  Ah, yes, that was an eddy!

We know not of its birthplace,
  Nor when it first saw day,
But Gründlingh has a theory:
  They're born off Richards Bay[3].
The coastline at St Lucia
  Deflects the Mighty Flow,
And southwards in its wake
  The eddies form and grow.

Yet that's not all the answer,
  For other data shows
The inshore flow is governed
  By the weather-bringing "lows"[4,5].
These cells of falling pressure
  Arrive from west-sou-west
And bring the dreaded "busters"[6]
  Which the mariners detest.

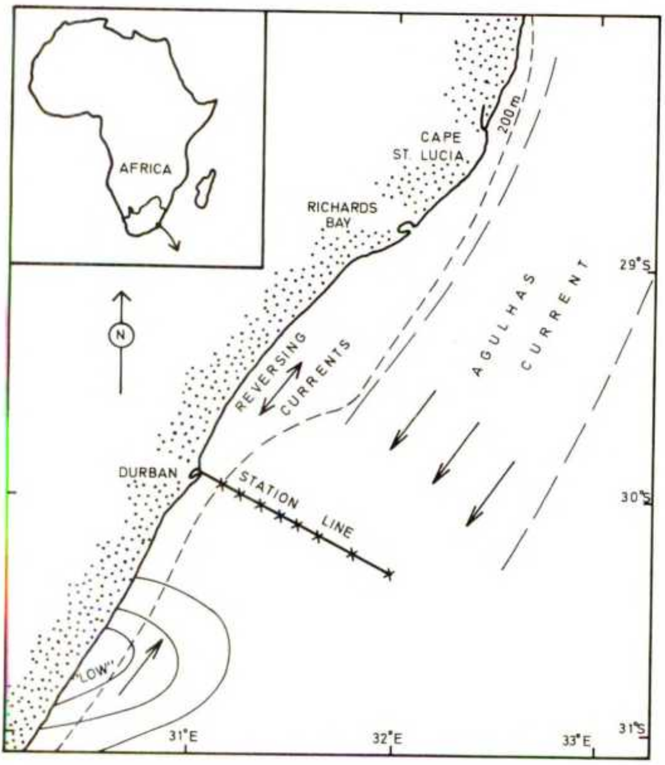


Fig. 1 Location chart, illustrating diagrammatically the general flow of the Agulhas Current, the reversing current on the continental shelf, the migration of a typical cyclonic cell from the south and the line of eight oceanographic stations. This line was occupied daily for the 5 d period February 19–23, 1973.

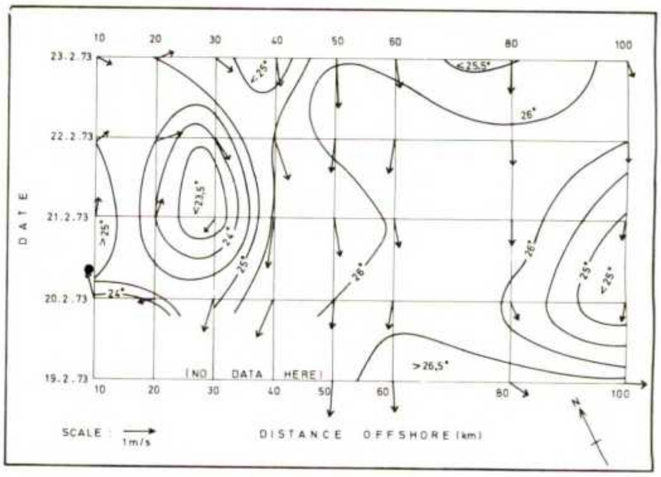


Fig. 2 The eddy is shown by the sea-surface temperature contours ($\frac{1}{2}$ °C intervals) and the current vectors measured at 10 m depth during the 5 d of the cruise.

And years of observation
  Along the eastern coast
Suggest that eddies form
  When pressure's down the most.
It therefore seems quite likely
  That instead of from the north
It's really from the south
  That the eddies issue forth.

Alas, our single section
  (Repeated occupation)
Did not show the direction
  Of eddy propagation.
Is it to north or south
  The eddies set their face?
Or maybe they are born
  and die in the same place[7]?

We're planning now a programme
  To show them in their glory.
We'll seek them—find them—track them—
  Until we know their story[8].

A. F. Pearce

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[1] See the location chart in Fig. 1.

[2] Fig. 2. A more detailed publication on this and other eddies associated with the Agulhas Current is in preparation (at present they are described in various unpublished reports).

[3] Gründlingh, M. L., *Deep-Sea Res.*, **21**, 47–55 (1974).

[4] Anderson, F. P., Sharp, S. O., and Oliff, W. D., *Symp. Oceanography in South Africa*, Durban, 1970, paper H2, page 22.

[5] Pearce, A. F., *South African National Oceanographic Symp.*, Cape Town, 1973, page 28.

[6] The "lows" move up the coast from the south at intervals of a few days, and bring in their wake south-westerly winds; when these are of gale intensity, they are termed "busters".

[7] It is of course possible that standing eddies may be generated by the sudden change in the 200 m isobath north of Durban – there is evidence of semi-permanent patches of cooler water off Durban, shown by airborne radiation thermometer surveys.

[8] Eddies of a similar (?) nature associated with the Gulf Stream system have been described by Lee, T. N., dissertation, Florida State Univ. (1972).

review article

Control of growth of mammalian cells in cell culture

From the Library,
Science College,
92 Upper Circular Road
Calcutta

Robert W. Holley*

Recent studies of the control of growth of mammalian cells are considered and it is concluded that the factors that control growth are probably polypeptide hormones, or hormone-like materials, as well as a variety of low molecular weight nutrients. An understanding of the control of growth of mammalian cells will probably be found in the complex interactions of cells with these common types of materials.

ORDERED, controlled growth is essential for the development and maintenance of a normal animal. The mechanisms that control growth are selective for different types of cells within an animal and are responsive to changes in the environment of cells, as, for example, after wounding.

Some aspects of growth control are observed in cell culture. In general, normal cells in culture show more restricted and ordered growth than their transformed or malignant counterparts. The extent to which growth controls are lost in cell culture parallels the tumorigenicity of the cells *in vivo* in some instances[1,2], suggesting that the growth controls seen in culture have relevance *in vivo*.

Two questions are of particular interest. What factors control the growth of normal cells? Why do the same factors not control the growth of transformed or malignant cells?

"Density dependent regulation" of growth

Normal, untransformed cells show "density dependent regulation", that is, they tend to grow to a certain "saturation" density and then stop growing. The resulting quiescent cells can remain both healthy and quiescent for some time. Highly transformed cells, in contrast, have lost "density dependent regulation" and they grow continuously until they exhaust the supply of low molecular weight nutrients in the medium; the cells do not become quiescent, and they must be transferred to fresh medium or they die. To understand the difference between normal and transformed cells, it would be helpful to know what factors control the growth of normal cells during "density dependent regulation".

Initially, "density dependent regulation" was ascribed to "contact inhibition"[3], in analogy with contact inhibition of movement of cells[4]. It was postulated that contacts between crowded cells repress further cell proliferation. Although the term "contact inhibited" is still used in the literature to describe quiescent cells, many lines of evidence indicate that quiescence is not caused by contacts between cells. Instead, arrest of growth of mammalian cells during "density dependent regulation" appears to be the result of limitation of any one of a variety of materials in the medium surrounding the cells.

Factors in the control of growth

In "density dependent regulation", cells grow to their "saturation density" and become quiescent. The "saturation density" varies with the type of cell and the culture conditions. Though few situations have been studied in detail, it is clear that many different factors can control growth in this way. The factors encountered in routine cell culture fall in to two broad classes. They may be either macromolecular factors in serum[2,5,6], or low molecular weight nutrients in serum[7] or the synthetic medium[8–11].

"Density dependent regulation" that involves macromolecular serum factors is of particular interest, and, for reasons that will be discussed below, emphasis in this review will be placed on serum factors. The growth control that is observed is, however, at least superficially, the same when growth is controlled by low molecular weight nutrients. The cells grow to a predictable cell density, and after growth stops the quiescent cells are found primarily in the G_1 or G_0 phase of the cell cycle[8,11]. Growth reinitiates if the limiting factor is added to the culture[8,11]. There is evidence that the cells are arrested at the same "restriction point" whether the limiting factor is a low molecular weight nutrient or a macromolecular serum factor[12]. One difference that is observed when cultures are limited by low molecular weight nutrients is that it is more difficult to maintain the cells both quiescent and healthy, presumably because the nutrients have essential metabolic roles in addition to their growth regulatory roles.

The conclusion that the growth of mammalian cells can be controlled by many different factors is strengthened by experiments which deliberately vary the culture conditions with a single type of cell For example, the growth of 3T3 cells, a mouse embryo cell line, is normally controlled by macromolecules in serum[2,5,13]. The "saturation density" is directly proportional to the initial serum concentration in the medium[5,13]. If the experimental conditions are varied, however, the growth of these cells can be controlled, at least to some extent, by any one of the following pure materials· fibroblast growth factor[14,15], epidermal growth factor[16], insulin[15,17], hydrocortisone[18], prostaglandin $F_{2\alpha}$[19],

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cyclic nucleotides[20], calcium ions[21], amino acids[11], phosphate ions[11], glucose[11], and proteolytic enzymes[22].

The list of growth-controlling factors becomes even longer and more varied if other mammalian cell types are included. The list includes, besides the general types of materials listed above, peptides[23,24], antigens[25], lectins[25], and lipopolysaccharides[25,26] The length and variety of this list suggest that the mechanism that controls mammalian cell growth is responsive to some sort of complex interaction among many factors.

Polypeptide factors probably control growth *in vivo*

Of the list of pure materials that control the growth of 3T3 cells, the two polypeptides, the fibroblast growth factor and insulin, are active at extremely low concentrations. The two act synergistically and their action is potentiated by a glucocorticoid[15], reminiscent of the action of prolactin, insulin, and a glucocorticoid, on the growth of mammary gland cells[27–29]. Because they are active at such low concentrations, and because they could interact specifically with cell surface receptors on selected cells, it seems likely that the polypeptide factors represent the important, natural macromolecular factors that control growth of mammalian cells *in vivo*. Consistent with this view is the extensive list of similar polypeptide factors. The list includes nerve growth factor[30], epidermal growth factor[31], colony stimulating factor[32–34], the somatomedins[35,36] and non-suppressible insulin-like activities[37], rat fibroblast growth factors S1 and S2[38], mesenchymal factor[39] and ovarian growth factor[40], as well as the many polypeptide hormones that are known to control growth of tissues *in vivo*. Similar factors, present in blood platelets, may be of significance in wound healing[41,42]. Certain of the polypeptide factors seem to be released into the culture medium by cell lines[32,37]. Numerous growth controlling factors probably remain to be identified.

Roles of other factors

If polypeptide hormones and similar growth-controlling serum factors have the key role in controlling mammalian cell growth, then what roles are played by the many other types of materials, listed earlier, that control cell growth in culture?

Many of these materials probably mimic hormone action. For example, proteolytic enzymes and other hydrolytic enzymes may, when they stimulate growth, act on hormone receptor sites[43], or they may alter the cell surface in ways that potentiate the action of polypeptide hormones Small peptides that are active as growth factors for some cell lines may also mimic hormone action. Exogenous cyclic nucleotides may affect growth by providing one of the products of polypeptide hormone action[44]. Antigens. lectins and lipopolysaccharides interact with lymphocyte cell membranes and, again, may activate cell proliferation by mechanisms that are similar to the actions of polypeptide hormones on the membranes of other cells.

Common low molecular weight nutrients may or may not act by the same general mechanism Low molecular weight nutrients seem to be able to regulate growth of most cells, suggesting that regulatory mechanisms involving such nutrients have important roles. Since similar mechanisms are observed in microorganisms, for example, in the "stringent" amino acid control of bacteria[45], these growth control mechanisms may be very primitive in origin. They may be important in animals in starvation conditions, and although they may be completely unrelated to the action of the polypeptide hormones and growth factors, an intriguing possibility is that the hormones and growth factors act by invoking the same intracellular growth control mechanisms affected by nutrients[46].

In addition to all the factors that stimulate growth, other polypeptide factors, and possibly "chalones"[47], may act to inhibit growth of cells. An example of the inhibitory action of a polypeptide hormone is the suppression of growth of adrenal tumour cells by ACTH[48].

Roles of polypeptide factors in "density dependent regulation"

Assuming that the polypeptide factors are the important growth-controlling factors, what is their role in "density dependent regulation" of growth? Two kinds of situation can be studied experimentally. Cells can be studied at different densities in different culture dishes, or, using special manipulations, they can be studied at different densities in the same culture dish.

With 3T3 cells, growth can be arrested at any cell density by choosing the appropriate serum concentration[13]. Whatever the cell density, initiation of DNA synthesis takes place in the quiescent cells if the concentration of serum or the pure polypeptide factors is raised. The higher the original quiescent cell density, the higher the serum or polypeptide factor concentration must be raised to initiate DNA synthesis.

The most dramatic demonstration of "density dependent regulation" involves making a "wound" in a quiescent, confluent cell layer by removing a narrow strip of cells[2,49,50]. Cells migrate in from the edge of the "wound", initiate DNA synthesis and divide, and cell proliferation eventually fills in the "wound"[2,49]. Proliferation of sparse cells within the "wound" takes place in the same medium in which crowded cells, in the confluent layer, remain quiescent

In the "wound healing" experiment with 3T3 cells it can be shown that serum factors are essential Without serum in the medium, cells at the edge of the "wound" do not migrate into the open area and the cells do not initiate DNA synthesis[50,51]. A fraction has been obtained from serum that promotes migration of 3T3 cells into the "wound" in the absence of serum, but DNA synthesis is not initiated in these sparse cells unless the macromolecular factors that control growth are also added[51]. The same factors initiate DNA synthesis in the sparse cells and in the crowded cells, but a lower concentration of factors is effective in sparse cells[51].

These results in the "wound healing" experiments with 3T3 cells suggest that the same macromolecular factors that control normal growth are the important factors in "density dependent regulation".

The major inconsistency in the literature is the report that certain epithelial cells have a negligible "wound serum requirement"[50], that is that these cells initiate DNA synthesis in the "wound healing" experiment in the complete absence of serum This suggests the possibility that epithelial cells show some other kind of growth control. This question has been reinvestigated using BSC-1 cells, an established line of African green monkey kidney cells. It has been found that the growth of BSC-1 cells, in either sparse or crowded culture, or in a "wound", is dependent on a low level of macromolecular serum factors (unpublished work). The earlier report that initiation of DNA synthesis takes place in BSC-1 cells at the edge of a "wound" in medium without serum seems to be due to difficulties in washing away all the serum factors when the cells have been grown to confluence in 10% serum.

It seems likely, therefore, that "density dependent regulation" is due to a quantitative increase in the requirements for macromolecular growth factors as cell density increases

Mechanism of "density dependent regulation"

It is not clear why crowded normal cells need a higher concentration of the macromolecular factors for growth. It is known that the polypeptide growth factors are destroyed by the cells[5]. Increasing the cell density probably increases the rate of destruction, and there is evidence that destruction of the factors creates a diffusion boundary layer[52]. In addition it seems likely that cells become less responsive to a given concentration of the growth factors as their surface area and movement decrease, perhaps because of a decrease in the number, availability or mobility of surface receptors for the factors. There is much evidence that the surface area of the cells[53–55] is important in "density dependent regulation".

Mechanisms of action of factors that control growth

Although there have been many studies of the initiation and arrest of cell growth in culture, little is known about the internal mechanisms that control cell growth. It is known that a complex set of changes follows the stimulation of growth of quiescent cells by hormones or serum, the "pleiotypic response" described by Tomkins *et al.*[56]. There are changes in transport of nutrients including ions[38,56–58], changes in intracellular concentrations of cyclic nucleotides[59–61], changes in RNA and protein synthesis[2,58], and changes in microtubular assembly[62] After 10–15 h, the initiation of DNA synthesis takes place. Because of the length of time involved, it seems likely that an extensive programme of events is required between the addition of the growth stimulus and the initiation of DNA synthesis. Since many new proteins are probably synthesised during this period, extensive changes in RNA and protein synthesis would be anticipated. It is not known whether the various facets of the "pleiotypic response" are under coordinated control[56], or whether all the observed changes are required to sustain the chain of events that leads to DNA synthesis For practical reasons, attention has been focused on the very early events.

The earliest changes that have been detected following growth stimulation are changes in intracellular concentrations of cyclic nucleotides[59–61] and changes in membrane permeability and transport of nutrients[38,56–58]. These very early changes are, however, characteristic of polypeptide hormone action in general[63]. That the changes occur supports the conclusion that hormone action is the natural mechanism of growth control, but, if one accepts this conclusion, observation of the changes tells little more

It has been proposed that cyclic nucleotides play the crucial role in growth control, with a decrease in the intracellular concentration of cyclic AMP[59,64] or an increase in the ratio of cyclic GMP to cyclic AMP[60,61] causing the initiation of growth. There seem, however, to be instances in which the initiation of growth is accompanied by changes in intracellular cyclic nucleotide concentrations other than those expected[65–67]. Also, a mutant cell has been obtained that is deficient in adenylate cyclase[68]. Since calcium ions seem to be intimately involved with cyclic nucleotides, it is possible that calcium ions are critical in what is observed[69]. There is also the possibility that the membrane permeability changes, caused by polypeptide hormone[63] or serum factor action, are themselves the crucial event in growth stimulation. These permeability changes may activate the same intracellular control mechanisms mentioned earlier that operate with low molecular weight nutrients[46].

Whatever hypothesis one favours, it seems likely that an understanding of the mechanisms of action of the factors that control mammalian cell growth will involve an understanding of the mechanisms of action of the polypeptide hormones.

Loss of growth controls in transformed cells

Transformed cells tend to grow to very high cell densities in cell culture. Under identical culture conditions, the growth of the corresponding normal or untransformed cells is limited by the supply of macromolecular serum factors in the medium. This suggests that transformed cells have a decreased requirement for the macromolecular serum factors and this decrease has been found. In some instances the transformed cells seem to have lost the requirements for the normal growth-controlling factors completely[13,70,71]. In other instances the transformed cells retain the requirements but at a quantitatively lower level than normal cells (ref. 1 and unpublished work).

The causes of the loss or quantitative decrease of the requirements for macromolecular growth-controlling factors are poorly understood. It is quite possible that the causes differ with different transformed cells. In one instance, studies of a transformed cell that retains a requirement for the normal serum factor show that a decrease in serum requirement to one-tenth the original value is accounted for by the combination of a decrease in the concentration of serum required to initiate DNA synthesis and a decrease in the rate of destruction of the serum factors by the cells (unpublished work).

Either of two basically different alterations might underlie the changes that lead to the lower requirement for serum factors. One possibility is that transformation alters internal regulatory mechanisms, leading to the direct loss of control of DNA synthesis. Alternatively, transformation may lead to a product that affects the cell membrane, and membrane properties may be altered in such a way as to modify the requirements for hormone interaction with the membrane.

Neither possibility can be excluded by present evidence. Some highly transformed cells seem to have lost essentially all growth controls, consistent with direct loss of control of DNA synthesis The growth of many transformed, tumorigenic cells can, however, be arrested easily in G_1 or G_0, and some transformed, tumorigenic cells have what seem to be modified hormone-cell surface growth controls. This seems more in keeping with the second possibility, that transformation affects growth control by changing the cell membrane. Membrane changes accompany transformation[72,73], but the membrane changes may be the result rather than the cause of changes in growth behaviour.

Though it is not posible at present to distinguish between the two possible mechanisms of altered growth control, many powerful approaches are now directed at this question. Studies of the actions of the gene products of tumorigenic viruses[74] and studies of the properties of isolated vesicles of plasma membranes of normal and transformed cells[75] are examples of approaches that may distinguish between the two possibilities and may lead to an understanding of the loss of growth controls that accompanies malignant transformation.

Summary

The overall picture that emerges is that growth of normal cells is probably controlled by interactions of the cells with various polypeptide hormones or hormone-like growth factors present in the surrounding fluids. As normal cells become more and more crowded they require higher and higher concentrations of the growth factors in order to grow. This increased requirement for growth factors is probably due in part to diminished surface area of individual cells when they are packed in among other normal cells, and in part to cellular destruction of the factors, which lowers their concentrations in fluids adjacent to crowded cells. More exotic possibilities, such as control of growth by "contact inhibition", have not received convincing support.

Transformed or malignant cells escape from normal growth controls by requiring less of the hormones or growth factors. The cells initiate growth in response to lower external concentrations of growth factors. They probably destroy the factors less rapidly, and they probably maintain more surface area exposed to the surrounding fluids.

The changes in transformed cells that lead to lowered requirements for the growth factors may be the result of direct internal loss of control of DNA synthesis or to membrane changes that modify the requirements for hormone interaction with the membrane.

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articles

Evidence for earthquake triggering stress

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Earthquake time series are constructed by making counts per unit time of earthquakes with magnitudes greater than a chosen threshold. The time series from widely separated regions show strong correlations with one another, and it is suggested that tectonic plates are subjected to a fluctuating stress field that results in these changes in seismic activity.

Two assumptions are commonly made about earthquakes: first, that their occurrence has a stationary random Poisson distribution and, second, that most earthquakes are essentially local phenomena. To question these assumptions we have compared the time variation of seismic activity at a number of locations throughout the world.

Very large earthquakes account for most of the energy release[1] and fault slip[2] within a seismic zone. One would expect such earthquakes to occur very systematically if the underlying plate motion is regular. In practice, however, the timing of large earthquakes at a given location seems to be almost random[3]. Spatially, evidence is accumulating that large earthquakes occur in sequence[4], around a plate margin. Migration velocities of several hundred km yr^{-1} have been attributed to the process of stress diffusion[5].

Earthquakes smaller than 'very large' do not contribute significantly to the motion of the plates adjacent to a seismic zone, and they are usually considered to be local phenomena. Most, one would presume, are either aftershocks, or some local reaction to the stress changes accompanying the infrequent major events. Their statistical characteristics are found to agree well with a generalised Poisson model[6]. The constancy of this process in time is, however, rather poorly understood. This is important, for it lies at the heart of methods for the estimation of earthquake risk.

Procedure

We are concerned primarily with earthquakes of small to medium size. The data used have been obtained from several earthquake catalogues. The primary data set is the list of earthquakes published (currently) by the National Earthquake Information Service of the US Geological Survey and commonly known as the PDE (Preliminary Determination of Epicentres) list. We have used this list for the period January 1, 1964 to April 30, 1974. Supplementing this, we have the Regional Catalogue of Earthquakes published by the Japan Meterological Agency (January, 1964 to January, 1970), the list[7] of earthquakes in the southern California region (January, 1964 to December, 1971), and the bulletin of the International Seismological Center (January, 1964 to December, 1970). The starting date of January, 1964 was chosen because several of these catalogues were rather incomplete before this date.

No attempt was made to remove aftershocks from these catalogues. We realise that this would be desirable, and attempts to do this are continuing. Some aftershocks are easy to identify, but in many cases it is not at all clear whether an earthquake is an aftershock or not. For the present, we prefer to change the data sets as little as possible.

To make our earthquake time series, we have extracted counts per unit time of earthquakes with magnitudes greater than or equal to a given threshold. The thresholds chosen were high enough to ensure that the catalogues were essentially complete, but low enough that a reasonable number of events were included. For example, in the case of the PDE listing, earthquakes with $m_b < 5.2$ were not used, since there are doubts about the completeness of this catalogue at $m_b \leqslant 5.0$. Note that the particular definition of magnitude used is immaterial in this representation (for example, the Southern California Catalogue uses local magnitude M_L).

Analysis

Changing this threshold upwards from its minimum value produced only minor changes in the character of the resulting earthquake series. We interpret this as follows: Within a region, the shape of the frequency–magnitude curve seems to be remarkably stable. Its level, however, changes significantly from one time interval to the next. If this relationship is approximated by a straight line

$$\log N = a - bM$$

where N is the number of events with magnitude $\geqslant M$, and a and b are constants, then b is found to change very little with time. On the other hand, a seems to undergo rapid fluctuations. Clearly, the time series we have constructed give an indication of the variation in the quantity a, which may be called the level of seismic activity.

The choice of the time intervals for the earthquake counts is a little more difficult. Wider intervals contain more events, but also appear to smooth out some high frequency components in the variation of seismic activity. Counts over 40-d intervals were used extensively, and an example is shown in Fig. 1*a*. In this case shallow (depth < 100 km) earthquakes from subduction zones bordering the Pacific Ocean with $m_b \geqslant 5.5$ are shown. Some structure is visible in this histogram, particularly in the period 1964–69, but it is still dominated by 'spikes' due to the aftershock sequences of a few large events. We have added more smoothing, by applying a sliding 200-d window to the data, and this is shown in Fig. 1*b*.

Figure 1*b* is not very surprising. The years 1964 and 1965 were dominated by the aftershocks of the Alaskan Earthquake (March, 1964) and Rat Island sequence (February, 1965). Activity lows of some significance occurred in mid-1966 and mid-1967, and then the activity returned to a reasonably uniform level for the remainder of the period. The true significance of this figure is not apparent until it is compared with the variations from smaller individual seismic zones.

For a start, let us compare shallow events with deep events. Figure 1*c* shows the variation for events with depth > 300 km, and $m_b \geqslant 5.2$, for the whole Pacific area. The depth of 300 km was chosen to ensure a complete separation of deep and shallow events. Remarkably, a high activity in 1964 and 1965 is again shown, falling off to extreme lows in mid-1966 and mid-1967. The remainder of the curve does not correlate in detail with the shallow events. Note that, this time, the 1964–65 activity high can not be attributed to the Alaskan and Rat Island events, since both were shallow. In fact, many of the events in Fig. 1*c* come from the Fiji–Tonga area.

Figure 2 shows smoothed earthquake time series for six regions bordering the Pacific Ocean. These have been compiled from the PDE catalogue, and contain only shallow events (depth < 100 km). The separation into regions was accomplished using the standard seismic region numbers[8]. If earthquakes really are local phenomena, we would expect essentially no correlation between these time series. Instead, we note that each time series has a high during 1965, though the location of the peak is somewhat variable, and this is followed by distinct activity lows in 1966 and 1967, as found for the Pacific as a whole (Fig. 1). An activity high in 1968 is apparent in most of the time series, and the correlation becomes rather low for the last five years of the record. Clearly, the general features of the first five years of the series shown in Fig. 1*b* appear in most of these localised regions.

One immediate explanation for these observations might be that they are a characteristic of the PDE catalogue. To check this, Fig. 3 shows the time series for six widely separated areas, using several earthquake catalogues. The areas concerned include subduction zones, mid-ocean ridges and a transform fault (southern California), and the general features of Figs 1 and 2 are again apparent. Notice in particular the remarkable correlation between the top three series of Fig. 3, for the period 1964–68.

Attempts to quantify the correlations between these time series have met with little success. Standard methods of cross correlation tend simply to match the largest peaks, and do not

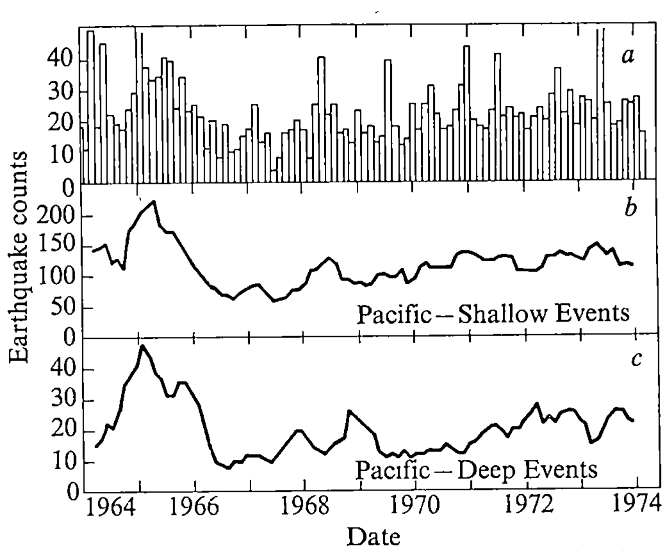


Fig. 1 Earthquake counts for the seismic zones bordering the Pacific Ocean, using the PDE catalogue. *a*, Histogram of shallow (depth < 100 km) earthquakes with $m_b \geq 5.5$ at 40 d intervals. *b*, Smoothed version of the histogram above, using a sliding 200 d window. *c*, Time series of deep (depth > 300 km) earthquakes with $m_b \geq 5.2$, for the same zone, smoothed as in *b*.

emphasise the features apparent to the eye. We are, however, accumulating evidence that the spectra of these time series have some common characteristics, and this will be discussed in a later paper.

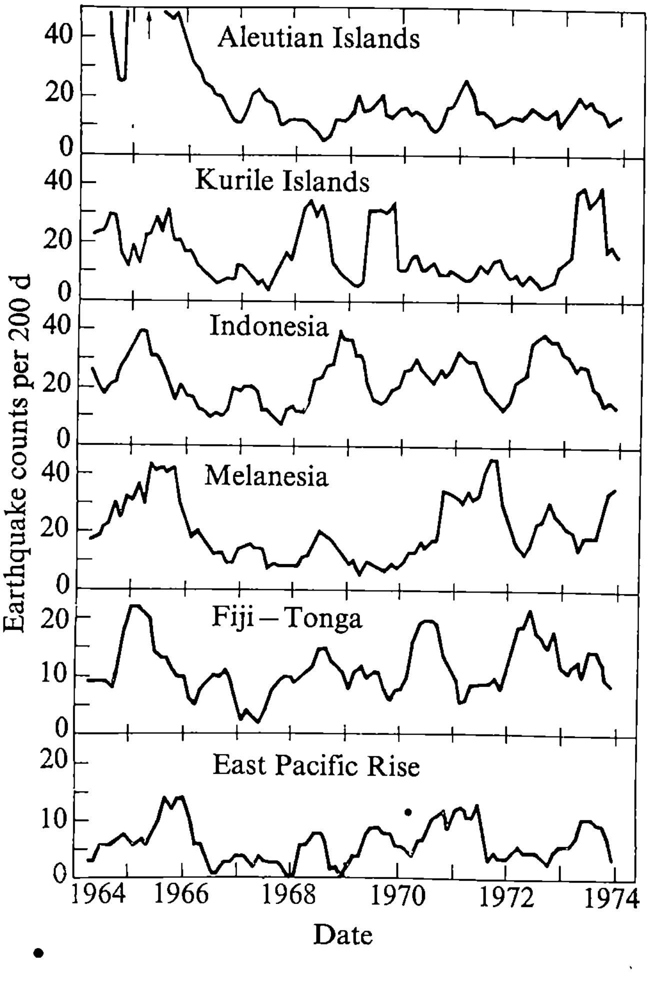
Conclusion

The conclusions from the data shown in Figs 2 and 3 are largely self-evident. Seismic activity, even of small earthquakes, contains significant variations in level that are correlatable over large distances, and between different tectonic environments. Viewed as a random process, seismic activity is highly nonstationary. The correlations shown must, however, be interpreted as indicating a strong non-random component.

If very high thresholds for magnitude are used, or if the data are summed to obtain energy release or fault slip, the correlations in Figs 2 and 3 are not apparent. In fact, the most pronounced feature of all, the activity high in 1965 followed by the low in 1966, is not apparent on these curves. We must conclude, therefore, that seismic activity and seismic energy release (or fault slip) are not the same thing.

We can account for these observations by invoking the concept of a triggering stress. We postulate that tectonic plates are subject to stresses of several different origins, with different amplitudes and spectral characteristics. There must be a long term stress accumulation due to the basic mechanism of plate motion, presumably as a result of processes in the Earth's interior. Superimposed on this, however, will be a variety of shorter term, often periodic, stresses. External effects such as changes in the rate of rotation of the Earth[9] and tidal stresses must be important here. The net sum of all these is a fluctuating stress that is generally of rather low amplitude (almost certainly $< 10^6$ Pa (1 bar)) but which will change in a complicated way.

Fig. 2 Earthquake time series for six regions on the margins of the Pacific Plate. Magnitude thresholds vary from $m_b=5.2$ to $m_b=5.6$. Source: PDE earthquake catalogue.



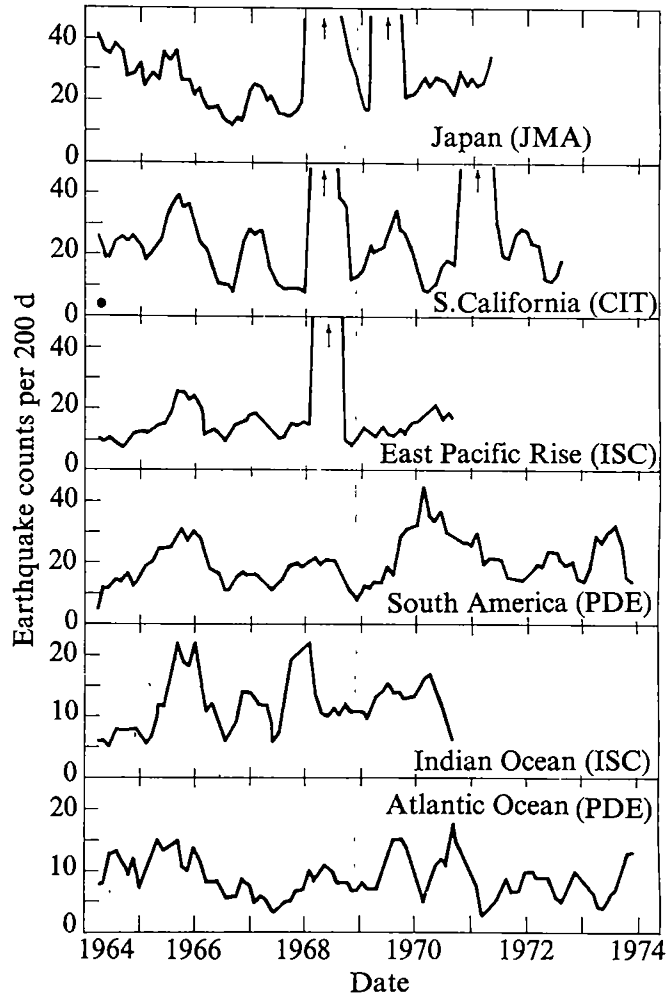


Fig. 3 Earthquake time series for six widely separated areas. Sources and magnitude thresholds are as follows: JMA, Japan Meteorological Agency Catalogue ($m_b \geq 5.2$); CIT, Southern California Network Catalogue ($M \geq 3.5$); ISC, International Seismological Center Bulletin ($m_b \geq 4.8$); PDE, PDE earthquake listing ($m_b \geq 5.2$).

Such a fluctuating stress may be expected to trigger an earthquake in any area where the ambient stress is very close to failure. In most cases, only small earthquakes will be triggered. Occasionally, however, the small increment in stress will trigger a large earthquake, though there may be little relation between the size of the stress change and the size of the triggered event. This is presumably the reason why seismic energy release seems to be a quasi-random process.

It is also conceivable that this triggering stress may very occasionally become quite large, when the various contributions to this stress happen to reinforce one another. At such a time one might expect a widespread triggering of seismic activity, including a number of large events. This is essentially the hypothesis that Anderson[9] has proposed to account for the strong increase in seismic activity that occurred about the year 1900. Perhaps a similar explanation may apply to other periods of unusual activity, such as the year 1755, when large earthquakes occurred in Central America, Persia, Iceland, Lisbon, Morocco and Switzerland, and New England experienced the largest earthquake in its historical record.

The implications of this hypothesis are important. If it is true, then to investigate the nature of the triggering stresses we should be studying the occurrence of small earthquakes, not large ones. The level of seismic activity, defined by the level of the frequency–magnitude curve, may be giving us a direct measure of this fluctuating applied stress. This may lead to a whole new approach to the problem of earthquake prediction.

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Evolutionary size increase and longevity in Jurassic bivalves and ammonites

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A study of the relationship between the rate of increase of size and generic or specific longevity in Jurassic ammonites and bivalves shows that taxa which increase more rapidly become extinct more quickly. The results, which can be interpreted stochastically, make it possible to distinguish two modes of evolution respectively involving an increase and decrease in size.

THERE is a common tendency recognisable in the fossil record for animal groups to have evolved towards larger size; it is generally known as Cope's Rule[1–3]. No systematic attempt has, however, been made to investigate any possible relationship between the rate of size increase for particular taxa and their duration in time, or longevity. I report here on a study of the relationship between size increase and longevity for two of the most abundant macroinvertebrate groups in the classic marine Jurassic deposits of western Europe, the bivalves and ammonites (see refs 4–10). Cope's Rule is well exemplified by both fossil groups. Full allowance has been made for ecological control of size in different facies, and there is no evidence of a secular environmental trend through the Jurassic which could have affected the size attained by particular taxa at successive horizons[11]. Consequently, there can be little doubt that the size changes represent a genuine evolutionary phenomenon.

A good unit to use in measuring the rate of size increase is the darwin, proposed by Haldane[12] as a unit of evolutionary rate. One darwin is strictly defined as a change of e in a million years, but Haldane treated this as practically equivalent to an increase or decrease of $10^{-3}/10^{3}$ yr, that is, a doubling or halving of size in 1×10^{6} yr. The latter definition is adopted here.

Results

Data for the best documented bivalve species and genera (more strictly, species groups within genera) are presented in Table 1, which records the maximum dimension attained by each taxon at the earliest and latest stratigraphic occurrence, to the nearest zone, together with the longevity in zones and a measure of the rate of size increase. (The average duration of a Jurassic zone is approximately 1×10^{6} yr (ref. 11).) The mean rate of size increase is 109 mdarwin (range 6–546), which accords well with the values established by Van Valen[13] in a general survey of invertebrates and protists. He found a range of 3–300, with most values clustering between 30 and 150. The longevity ranges from 3 to 60 zones, the latter range representing almost the whole duration of the Jurassic Period.

For the ammonites, which have been excessively split taxonomically, the genus is generally the lowest reliable taxonomic unit, but Table 2 includes data for a few well defined species. The excessive splitting means that certain closely-related forms, which in other groups would almost certainly be put in the same genus, are given different generic names. For that reason several examples, for instance, *Grammoceras–Phlyseogrammoceras*, are included in Table 2. Because the stratigraphic range of most genera is so short adequate data are more difficult to obtain than for bivalves, so the number of examples is less.

There is little doubt that evolutionary size increase is more common than indicated by Table 2. For instance, Zeiss[14] records it as a common trend in Tithonian ammonities, though he fails to provide usable data. Because of the short time range size data have been determined where possible down to subzonal level. The mean value of 584 mdarwin, though significantly higher than that for the bivalves, is still probably too low, because the data are somewhat biased in favour of long-ranging taxa such as the oppeliid genera *Glochiceras*, *Ochetoceras* and *Taramelliceras*, which have relatively low rates of size increase. Whereas the zonal range is 1/3–15, the great majority of Jurassic ammonite genera have a stratigraphic range of about three zones or less.

When S, the rate of size increase, is plotted against L, the longevity, a strongly curvilinear relationship is exhibited for the two groups. Where the results are replotted logarithmically, the points cluster closely about a straight line (Fig. 1). Ammonites and bivalves, genera and species, large and small fossils do not form discrete groups but overlap in their distribution. It would give a spurious air of accuracy to calculate a regression and correlation coefficient, because the time scale is, of necessity, only approximate. It is, however, evident that the correlation is good and a regression line can easily be fitted by inspection. This indicates a relationship expressed by the equation

$$S = bL^{-a}$$

where a is the slope and b the intercept. The slope, a, is almost unity, and, if S is expressed in darwins, b is exactly unity. Therefore, the relationship between the ratio of size increase and longevity closely approximates to a hyperbolic function, $S = 1/L$.

This equation can be applied to the one result which cannot be plotted on the logarithmic scale, the ammonite *Metophioceras conybeari*, which has the shortest range and highest millidarwin value of all. The data for this species are based on examination in the field and can be considered relatively reliable. The rate of size increase, S, = 3.69 and $1/L$ = 3.33, which are satisfyingly close. The ammonite genus *Pleuroceras* has an abnormally high millidarwin value for its longevity of one zone. This could be attributable to several factors. The zone in which it occurs could have been of shorter than average duration, the minimum and maximum sizes may not have been separated in time by the whole duration of the zone, or the minimum

Table 1 Bivalve data

Taxon	Age range	Number of zones	Increase in maximum size (mm)	mdarwin
Astarte elegans (1)*	M.Toar.–L.Baj.	13	26–62	92
A. gueuxi (1)	U.Het.–L.Plien.	8	20–38	119
Barbatia pectinata (1)	L.Baj.–U.Oxf.	21	42–60	34
Camptonectes Iohbergensis–lamellosa (1, 3)	L.Plien.–Tith.	55	23–109	43
Cardinia concinna (1, 2)	U.Het.–U.Plien.	11	45–157	159
Ceratomyopsis striata (1, 2, 4)	U.Bath.–L.Kim.	18	58–90	43
Chlamys textoria (1, 2)	L.Sin.–U.Plien.	10	28–88	157
C. (*Radulopecten*) *fibrosa–inaequicostata* (1, 2)	L.Bath.–U.Oxf.	14	37–60	58
Coelopis lunulatus (1)	U.Toar.–L.Baj.	6	32–79	206
Ctenostreon tuberculatum–pectiniforme (1, 2, 3)	L.Sin.–U.Tith.	60	58–212	30
Entolium lunare (1)	U.Sin.–U.Plien.	8	34–110	202
Eocallista antiopa–brongniarti (1, 3, 4)	U.Bath.–L.Kim.	18	28–90	89
Eonavicula minuta–quadrisulcata (1, 3)	U.Bath.–L.Tith.	23	21–69	71
Eopecten velata (1)	U.Het.–U.Plien.	11	102–153	68
Eopecten abjecta (1, 2)	U.Aal.–U.Oxf.	24	75–112	31
Gervillella lanceolata–aviculoides (1, 2)	L.Sin.–U.Oxf.	40	60–262	55
Goniomya hybrida–literata (1)	U.Plien.–U.Oxf.	33	48–90	28
Grammatodon insons (1)	L.Sin.–L.Plien.	8	19–56	187
G. insons–oblonga (1, 3)	L.Sin.–L.Baj.	20	19–106	83
Gryphaea arcuata–gigantea (1, 3)	U.Het.–U.Plien.	11	60–125	90
G. bilobata–dilatata (1, 2)	U.Toar.–M.Oxf.	26	70–137	40
Isocyprina menkei–dolabra (1)	U.Sin.–L.Baj.	19	21–56	70
Isognomon lugdunensis–mytiloides (1, 2)	U.Plien.–L.Kim.	40	67–180	34
Liostrea irregularis (1)	L.Het.–U.Plien.	13	30–133	116
Modiolus hillanus–imbricatus (1)	L.Het.–L.Baj.	25	45–150	67
Myoconcha decorata (1)	L.Sin.–U.Plien.	10	51–108	106
M. decorata–crassa (1, 3)	L.Sin.–L.Baj.	21	51–145	68
Myophorella signata–clavellata (1, 2)	L.Baj.–L.Kim.	28	91–145	28
Palaeonucula navis–hammeri (1, 3)	L.Plien.–Aal.	11	8–29	165
Parallelodon buckmani (1)	L.Het.–L.Plien.	10	24–41	85
P. hirsonensis (1)	U.Toar.–L.Baj.	6	45–100	180
Pholadomya ambigua (1, 2)	L.Sin.–U.Plien.	10	68–150	110
P. lirata–protei (1, 3)	L.Baj.–U.Oxf.	22	78–108	31
Plagiostoma gigantea–hersilia (1, 2)	L.Het.–L.Baj.	21	100–207	39
Plicatula laevigata (1)	L.Plien.–U.Plien.	3	63–85	225
Propeamussium pumilum–laeviradiatum (1)	L.Toar.–L.Aal.	6	8–30	313
Protocardia phillipiana–dissimilis (1)	U.Sin.–L.Tith.	56	16–90	16
Pseudomytiloides dubius (1, 3)	L.Toar.–U.Toar.	5	30–45	150
Pseudopecten aequivalvis (1, 3)	L.Plien.–U.Plien.	3	61–200	546
Ryderia graphica (1)	L.Sin.–L.Plien.	8	14–38	170
Thracia depressa (1, 2, 4)	U.Toar.–U.Kim.	40	40–94	29

* Numbers in parentheses indicate location of specimens measured: (1), British Museum (Natural History); (2), Museum National d'Histoire Naturelle, Paris; (3), Palaentological Institute of Tübingen University; (4), Palaeontological Institute of Gottingen University.

Jurassic stages: Het., Hettangian; Sin., Sinemurian; Plien., Pliensbachian; Toar., Toarcian; Aal., Aalenian; Baj., Bajocian; Cal., Callovian Oxf., Oxfordian; Kim., Kimmeridgian; Tith., Tithonian. L., Lower; M., Middle; U., Upper.

Table 2 Ammonite data

Taxon	Age range	Number of zones	Increase in maximum size (mm)	mdarwin
Arnioceras semicostatum (1)*	L.Sin.	4	55–91	207
Aulacostephanus[10]	L.Kim.	3	175–345	328
Caenisites–Asteroceras (1)	Sin.	2	147–270	459
Cardioceras (1)	L.Oxf.	2	137–210	383
Coroniceras–Primarietites (1)	L.Sin.	1	350–650	928
Creniceras dentatum	L.Kim.	3	20–37	308
Garantiana–Parkinsonia (1)	U.Baj.	2	110–350	785
Glochiceras (3)	U.Oxf.–U.Kim.	10	39–56	64
Grammoceras–Phylseogrammoceras (1)	U.Toar.	2	114–194	425
Idoceras (3)	U.Oxf.–L.Kim.	5	154–221	143
Kosmoceras (*Gulielmites*)[4]	M.Cal.	1	70–150	1,071
Leioceras[7]	Aal.	1	60–100	833
Liparoceras[8]	L.Plien.	3	88–285	540
Metophioceras conybeari (1)	L.Sin.	1/3	130–320	3,690
Ochetoceras (3)	U.Cal.–U.Kim.	15	79–134	57
Pectinatites[5]	L.Tith.	3	184–380	206
Pleuroceras[6]	U.Plien.	1	66–335	2,538
Psiloceras (1)	L.Het.	1	130–253	973
Taramelliceras (1, 3)	L.Oxf.–L.Kim.	12	50–196	165

* Numbers in parentheses as for Table 1.

value, may be an underestimate, either because of inadequate sampling or because of a failure of preservation of the outer body chamber, which is not unusual among ammonites. These points only serve to emphasise the importance of very careful assessment of the evidence by specialists, and do not detract from the overall close correlation exhibited by Fig. 1.

That one parameter is so near to the reciprocal of the other is a remarkably simple result, especially bearing in mind the approximation of the time scale. The result is only in part an artefact of the method of plotting. By necessity the estimation of S involves using the reciprocal of L, but the amount of size change in time is an independent variable (in other words, the plot is not simply of one parameter against its reciprocal). If there were no such simple relationship as claimed between S and L there would be a much wider scatter of points in the graphical plot. The size increase that has taken place within individual taxa can be considerable. Thus a majority, almost the same in both groups (69% of bivalves, 68% of ammonites) have increased in size by a factor of approximately two or more, and a significant minority have trebled, quadrupled or even quintupled in size. On the other hand, a size increase of appreciably less than twofold is readily recognisable in the stratigraphic record.

Another possible criticism is that the results portrayed are selective, because they relate only to a minority of taxa in the European Jurassic. Though they are admittedly restricted to those taxa which seem to exemplify Cope's Rule, further work on abundant and precisely dated material may well show that the rule applies more widely. This is especially true for the ammonites, where a neglect of the significance of size differences has in the past influenced the classification of taxa, as is evident from the widespread failure until recently to recognise sexual dimorphism, with different names being given to the macroconchs and microconchs.

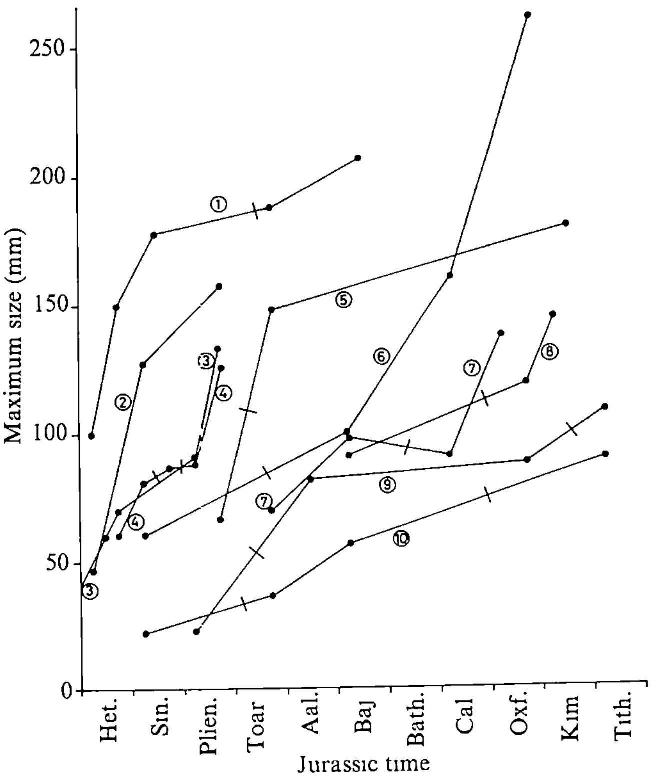

Fig. 2 Pattern of size increase during the Jurassic period for selected bivalves (stage abbreviations as in Tables 1 and 2). 1, *Plagiostoma;* 2, *Cardinia concinna;* 3, *Liostrea irregularis,* 4, Liassic *Gryphaea,* 5, *Isognomon;* 6, *Gervillella;* 7, Middle–Upper Jurassic *Gryphaea,* 8, *Myophorella;* 9, *Camptonectes;* 10, *Protocardia.* Cross bars indicate change of species or subspecies.

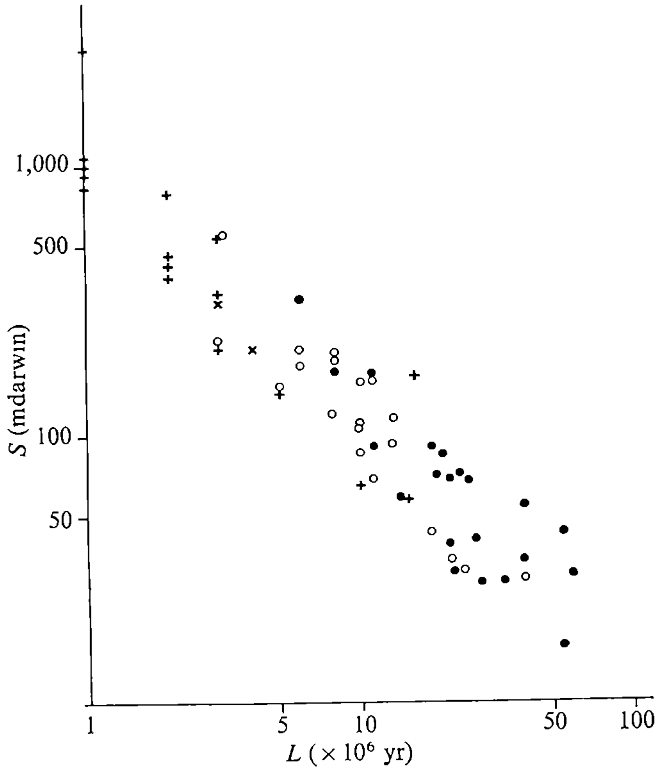

Fig. 1 Logarithmic plot of rate of size increase, S, against longevity, L, for Jurassic bivalves and ammonites. +, Ammonite genera; ×, ammonite species; ●, bivalve genera; ○, bivalve species

Before an interpretation is attempted some more facts should be considered. Figure 2 shows the nature of the size increase for a sample of longer-ranging bivalves where several data points are available, to illustrate the range of variation in pattern. Some forms (*Plagiostoma, Isognomon, Cardinia*) show a decrease in rate of increase with time, others an increase (*Gervillella, Myophorella*), or something close to a linear relationship (*Protocardia*). Others are more complicated, as, for instance, *Gryphaea*, for which there are more data available^{15,16}. The early Jurassic evolutionary lineage *G. arcuata–mccullochii–gigantea* shows successively a decrease in rate followed by an increase, whereas the Middle to Upper Jurassic lineage *G. bilobata–dilatata*, starts at a much smaller size than the end member of the older lineage, then increases, then decreases slightly, and finally increases again. These changes in rate of size change and absolute size correlate with taxonomic change, the new taxa, species or subspecies, sometimes being smaller than the taxa from which they evolved.

This illustrates an important principle; in both bivalves and ammonites younger taxa are often appreciably smaller than their evolutionary precursors, but with no discernible size transitions from one to the other. Good examples are, among the bivalves, the genera *Entolium, Gryphaea, Liostrea, Plagiostoma, Pholadomya* and *Eopectena;* among the ammonites the lineages *Psiloceras–Waehneroceras–Schlotheimia, Amaltheus–Pleuroceras,* and *Quenstedtoceras–Cardioceras–Amoeboceras.* A further point to note is that, because of adaptive radiation, both small and large species of the same genus may coexist in the younger part of the stratigraphic range. Close analysis is required, by ammonite specialists in particular, of whole phyletic lineages in uncondensed stratigraphic successions, in terms of times of morphological divergence and progressive size change. Although generally the ammonite genera may exhibit Cope's Rule, in some cases only the species may show it, assuming they are sufficiently well defined (compare with *Gryphaea*).

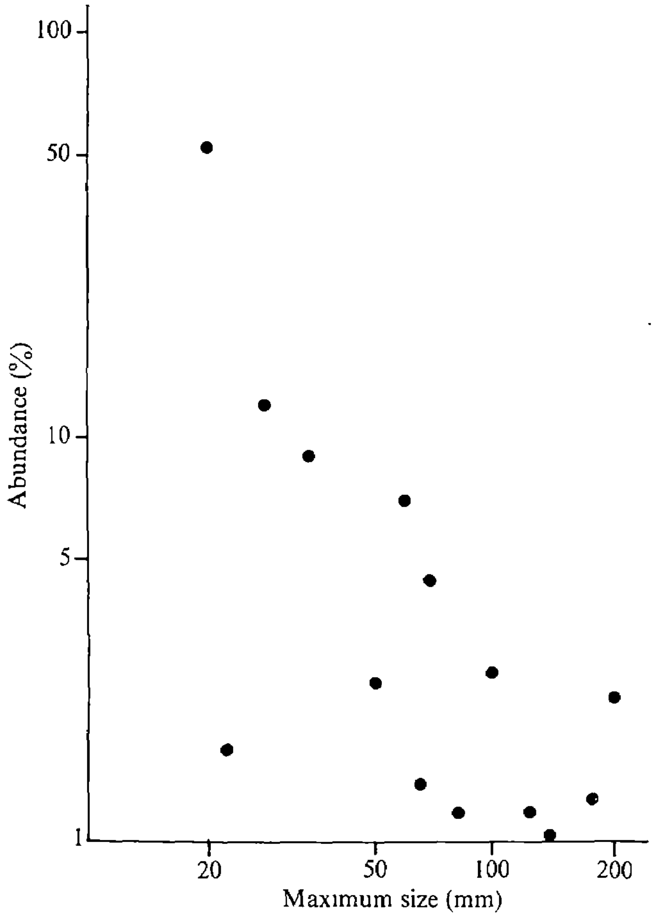

Fig. 3 Logarithmic plot of maximum size against abundance for the 14 most abundant species in the English Corallian Group.

Interpretation

Though ammonites and bivalves have evolved at markedly different rates, probably because of varying intensities of interspecific competition[17], they seem to obey the same rule relating rate of size increase to longevity. Since phyletic size increase is such a widespread trend in the animal kingdom there must manifestly be one or more selective advantages of larger size. Among those proposed[1–3] are: an improved ability to capture prey or ward off predators; a greater reproductive success; increased intelligence (with greater brain size); increased regulation of the internal environment; decreased annual mortality; and increased heat regulation per unit volume. Odum[18] considers that what he calls antithermal maintenance is the main priority in any complex system such as an organism, and this is decreased per unit of biomass structure if the individual's size is large. Stanley[3] considers that Cope's Rule should be viewed in terms of the evolution of small forms towards a larger optimum size for the animal group in question. It is not counterbalanced by an 'anti-Cope's Rule' because large animals of a given group are almost invariably too highly specialised to give rise to new forms, whereas many small animals are relatively unspecialised.

The acceptance of Stanley's interpretation of Cope's Rule implies that organisms that increase in size more rapidly should become extinct more quickly because extreme specialisation would occur earlier, thus rendering them more vulnerable to extinction. This may be termed the deterministic hypothesis which, though it may well be true in particular cases, suffers from the drawback that it assumes what requires to be proved. Too little is known of the mode of life of the extinct ammonites, or even the bivalves for that matter, for such an hypothesis to be accepted without considering an alternative.

Another, probabilistic, hypothesis can, however, be proposed. We may accept that selection pressures will tend to promote size increase, at least up to an optimum for a particular group, because of the manifest advantages already outlined. A 'price', as it were, has to be paid, however. Because food resources are likely to remain approximately constant, population sizes must decrease, thereby increasing the probability of extinction. Thus organisms that increase in size more rapidly should become extinct sooner. (The assumption that rarer animals are more likely to become extinct has received full theoretical treatment by McArthur and Wilson[19,20].)

The proposition that larger animals are rarer than smaller is abundantly supported by common observation. It is just as true within a particular group as between for instance, elephants and flies. Jurassic bivalves, for instance, exemplify the relationship well. Fürsich (personal communication) has made a systematic collection from all facies types of specimens from the English Corallian Group (Oxfordian Stage). Figure 3 shows a double logarithmic plot of abundance against maximum size for the 14 most abundant species (a total of 13,355 specimens). The logarithmic plot shows a closer resemblance to a linear relationship than a direct plot, which is suggestive of a power function. The scatter is inevitably considerable, however, which is not at all surprising. It would only be negligible if the relative abundance for a given size were constant, and there is no reason why that should be so. A best fit curve would have the advantage, in fact, of enabling one readily to distinguish forms which were more or less abundant than average for their size. The important point to be stressed is that these results give quantitative expression to the marked decline in numbers with increasing size. The smallest species, *Nanagyra nana*, accounts for 52% of the total number of individuals whereas the five largest species account together for a mere 8%.

The extraordinary, and unexpected, simplicity of the relationship between the rate of size increase and longevity, bringing under one umbrella large and small species and genera of two biologically very different molluscan groups, provides support for the stochastic interpretation.

In conclusion, two distinct modes of evolution can be proposed. One is more or less gradualistic and involves increase in size independent of changes in environment, which is generally speaking an evolutionary dead end, since it leads to eventual extinction, commonly without further phenotypic modification. It is a continuous unilinear trend involving orthoselection. The other involves the abrupt appearance of descendant species smaller than their ancestors: I know of no examples of gradual size decrease recognisable in the stratigraphic record, either in Jurassic molluscs or any other fossil group. Yet, undoubtedly, small organisms have evolved from larger, but the process must have been relatively sudden and probably involved small populations, so that the chances of finding intermediates in the fossil record is minimal. The process probably often involves paedomorphosis, and may be the principal means by which new morphotypes arise.

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Molecular structures of cytidine-5'-diphosphate and cytidine-5'-diphospho-choline, and their role in intermediary metabolism

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The nucleotide coenzyme cytidine-5'-diphospho-choline is highly folded. The CMP-5' parts of the molecules in the crystal structure are strongly linked by metal ligation and hydrogen bonds leaving the phosphoryl-choline residues relatively free. Cytidine-5'-diphosphoric acid exists as a zwitterion with N31 protonated. The P–O bond lengths from the anhydride bridging oxygen in the pyrophosphate are significantly different.

NUCLEOSIDE-5'-DIPHOPHATES have an essential role in many metabolic processes and are the starting substances for the biosynthesis of a variety of activated compounds of the type base–ribose–P–O–P–base (or sugar) involved in the synthesis of many key biological molecules. The presence of the pyrophosphate linkage, important for the activity of many 'high energy' molecules adds further significance to the structures of these molecules. No nucleoside diphosphate structure has, however, been reported so far, probably because of difficulties in growing crystals suitable for single crystal X-ray analysis. (A short report on the rubidium salt of ADP has appeared; F. A. Muller and A. B. Deluke, ACA Meeting, 1971; no structural details are, however, available.) To date the only nucleotide with pyrophosphate linkages to have been solved is the sodium salt of ATP[1]. We have now crystallised several of these compounds and have begun a systematic study of their conformations by X-ray analysis. Conformational differences among these are of interest as they behave differently in similar biochemical reactions. The group transfer reactions involving UDP sugars, for example, occur with the cleavage of the glucosyl–phosphate bond, whereas those involving CDP occur with the cleavage of the pyrophosphate linkage. X-ray studies of these molecules can be expected to provide information on those structural features of the molecules which relate to their biological function just as the structural studies of nucleoside-5'-monophosphates have now given a wealth of information relevant to the conformation of nucleic acids. Here we present the results of our study on the structures of cytidine-5'-diphosphoric acic (CDP) and cytidine-5'-diphospho-choline. CDP derivatives are key intermediates in the metabolism of phospholipids. CDP-choline, in particular, is involved in the biosynthesis of lecithin and sphingomyelin and is also important in the formation of plasmologen in liver and brain[2].

The two structures show several interesting features. The molecular parameters which contribute to the structural integrity of nucleoside-5'-monophosphates, namely the *gauche–gauche* conformation about the C4'–C5' bond and the *anti* conformation about the glycosidic linkage are preserved in the nucleoside-5'-diphosphates. The pyrophosphate orientations with respect to the furanose ring are, however, strikingly different and are related as right and left rotations about the terminal ester bonds of the pyrophosphate diester bridge. The CDP-choline molecules are highly folded and bind to each other so that the CMP-5' parts are rigidly linked by metal ligation and hydrogen bonds. In contrast, the phosphoryl choline groups are loosely held in the crystal structure by water molecules. Such association of coenzyme molecules may influence enzymatic reactions where the phosphoryl choline moiety is transferred leaving behind CMP-5'. The present structures provide for the first time the detailed geometry of the diester pyrophosphate linkage essential for the activity of many other important nucleotide coenzymes like UDP-glucose, nicotinamide adenine dinucleotide (NAD), flavine adenine dinucleotide (FAD) and coenzyme A, the X-ray structures of which have not yet been determined. On the basis of this geometry it now seems possible[3] to put forward rational molecular models for some of these molecules.

Experimental details

The CDP-choline used in this investigation was obtained as a monosodium salt from Sigma and the CDP free acid from Boehringer Mannheim. The crystals were grown by slow diffusion of acetone–alcohol mixtures into aqueous solutions of the samples, a technique found successful earlier in growing crystals of nucleoside-5'-monophosphates[4]. Crystallisation was carried out at about 5 °C to avoid possible breakdown of samples by hydrolysis. The CDP-choline crystals were predominantly platy and cleaved easily when allowed to dry fast. They were also strongly hygroscopic. All X-ray measurements were carried out on crystals sealed inside Lindemann capillary tubes along with mother liquor. In contrast CDP free acid grew as long needles and was quite stable in air. Cell constants were initially measured from Weissenberg photographs and subsequently on a Picker 4-circle diffractometer (Table 1).

The X-ray intensities were measured on a diffractometer, using copper Kα radiation, up to a 2θ maximum of 127° giving a resolution of 0.86 Å. There was no significant

Table 1 Crystal data

	CDP-choline	CDP free acid
Molecular formula	$C_{14}H_{25}O_{11}N_4P_2Na5H_2O$	$C_9H_{15}N_3O_{11}P_2H_2O$
Molecular weight	601.4	421.2
Space group	$P2_12_12_1$	C2
Z	4	4
a	29.434 Å	27.166 Å
b	12.436 Å	5 566 Å
c	7.016 Å	10.742 Å
β	—	99.99°
Volume	2568.15 Å^3	1599.63 Å^3
d_{measured}	1 57 g ml^{-1}	1.745 g ml^{-1}
$d_{\mathrm{calculated}}$	1.56 g ml^{-1}	1.749 g ml^{-1}
μ(CuKα)	43.6 cm^{-1}	30.1 cm^{-1}

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decrease in the intensities of monitor reflections over the course of the data collection (about 2 weeks). The total number of significant independent reflections was 1,398 for CDP and 1,439 for CDP-choline, to which the usual corrections other than absorption were applied. Full experimental details will be published elsewhere

Solution of structure

The positions of phosphorus atoms in both structures were located from sharpened Patterson maps and Harker sections. The structure of CDP was solved by the application of vector superposition techniques and difference Fourier syntheses as direct methods were not successful. Application of magic integers[5] and tangent refinement (locally programmed by Dr G. M. Sheldrick) aided the complete solution of CDP-choline. The structures were refined by full-matrix least squares calculations with anisotropic temperature factors to current R factors of 9.1% (CDP-choline) and 6.3% (CDP). The contributions from hydrogen atoms associated with the nucleotide molecules were included but water hydrogens could not be located due to high temperature factors of the water oxygen atoms.

Description of structures

The chemical structures and the numbering schemes adopted for the two compounds are shown in Fig. 1.

Nucleoside geometry. The orientation of the cytosine base with respect to the sugar is *anti* in both molecules, the glycosidic torsional angle χ_{CN} about the C1′–N1 bond being

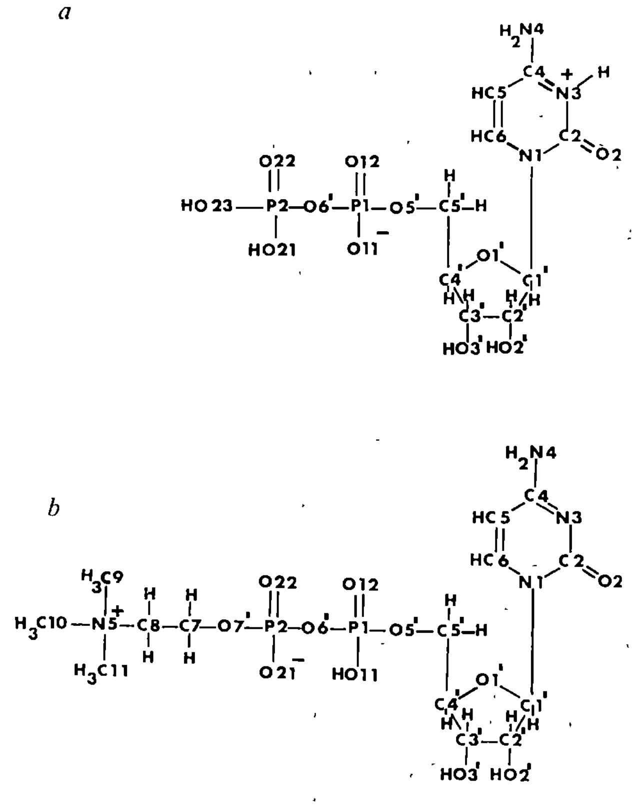

Fig. 1 Atom numbering scheme for: *a*, CDP; *b*, CDP-choline.

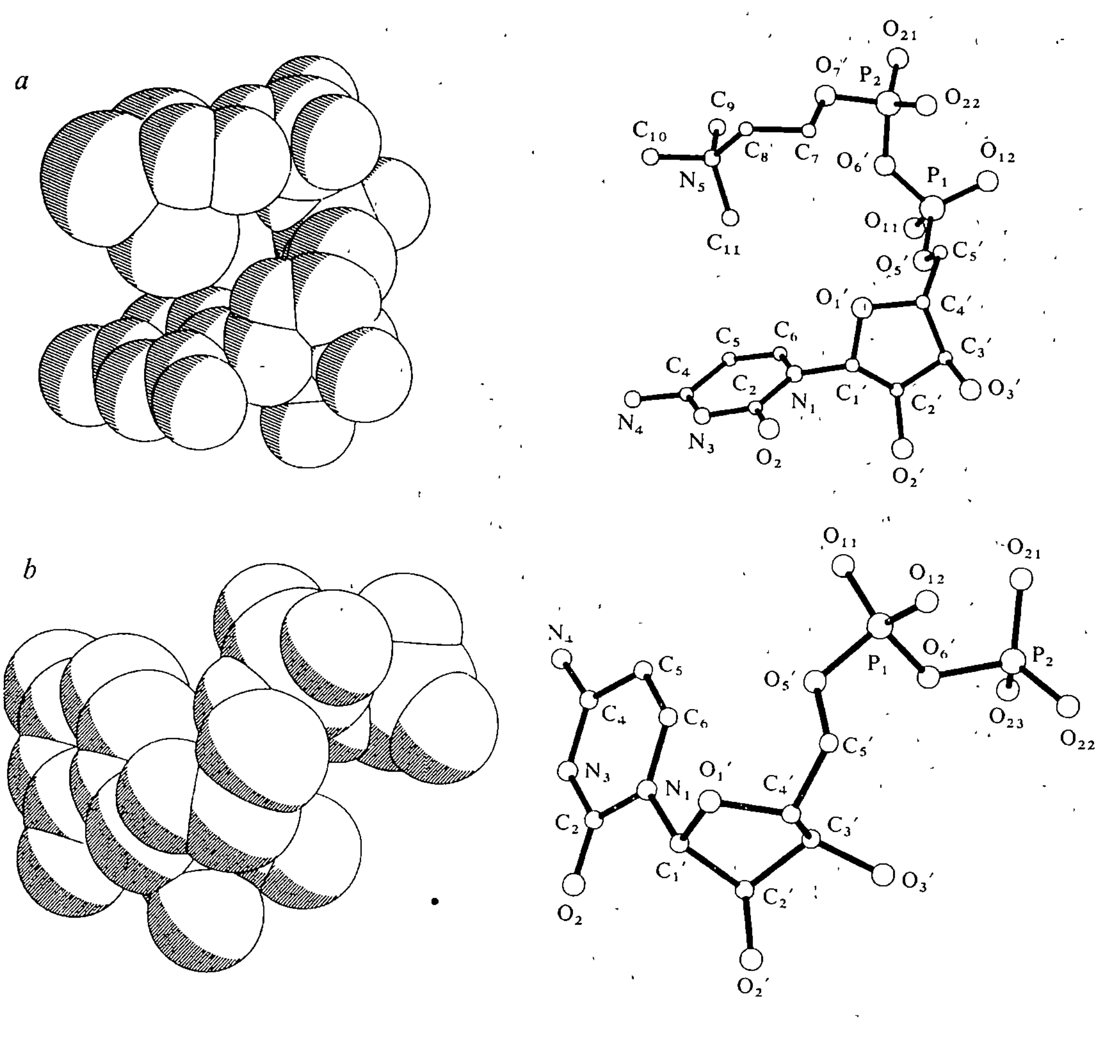

Fig. 2 Space filling and perspective drawings for: *a*, CDP-choline; *b*, CDP.

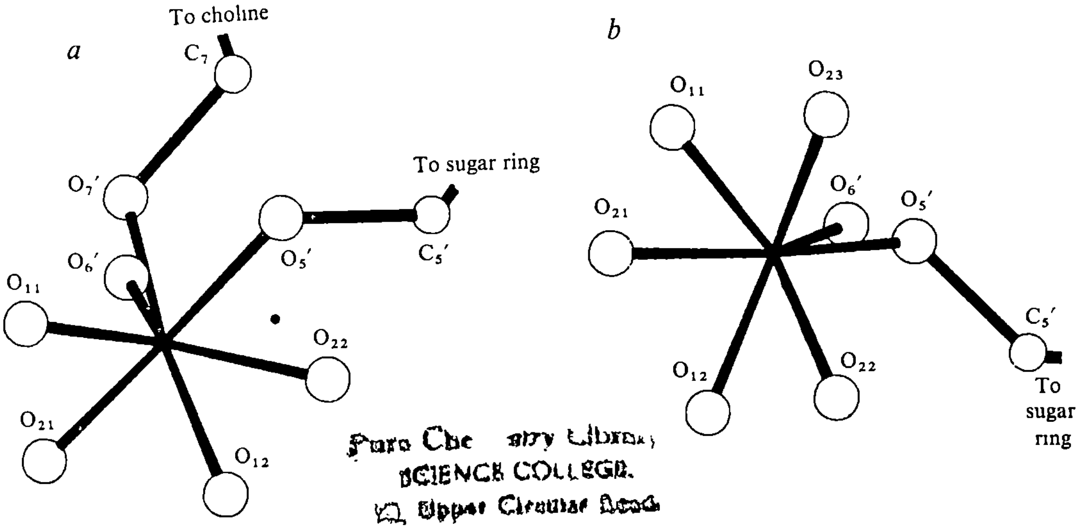

Fig. 3 View down the P–P vector: *a*, CDP-choline; *b*, CDP.

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22.9° (CDP) and 60.35° (CDP-choline), (for notations see Sundaralingam[6]). The ring nitrogen N3 in the free acid is protonated orming a cytosyl cation, due to single proton migration from the phosphate group. Earlier structure determinations of cytidine-3′-phosphate[7,8] and deoxycytidine-5′-phosphate[9] have established that cytosine nucleotides prefer the zwitterion structure. The bond lengths and angles of the base in CDP and CDP-choline correspond to those one expects for N3 protonated and neutral cytosine bases, respectively[9].

In CDP, the best four atom plane through the furanose ring passes through C4′–O1′–C1′–C2′. The C3′ atom is displaced from this plane by 0.63 Å on the same side as C5′. The sugar conformation is therefore C3′-*endo*. In CDP-choline, however, the sugar shows a typical C2′-*endo* puckering with C2′ displaced from the four-atom best plane by 0.57 Å. The distribution of glycosyl torsional angles χ_{CN} and their relationship to the nature of ribose puckering in the known *β*-pyrimidine glycosides shows[9,10] that C2′-*endo* puckering favours $36° \leq \chi_{CN} \leq 65°$ whereas C3′-*endo* puckering favours $0° \leq \chi_{CN} \leq 42°$. Note that the steric relationship observed in the nucleosides and their monophosphates is also the one found in the present diphosphate structures. In CDP-choline the cytosine base is almost perpendicular to the sugar ring, the dihedral angle between the normals to their mean planes being 92.81°. This angle in CDP is 107.02°. For each compound the conformation about the exocyclic C4′–C5′ bond is *gauche* with respect to both C4′–O1′ and C4′–C3′ bonds with the torsional angles: O1′–C4′–C5′–O5′=−60.18° (CDP) and −63.55° (CDP-choline) and C3′–C4′–C5′–O5′=55.9° (CDP) and 57.44° (CDP-choline). An earlier survey of X-ray crystallographic results has led Sundaralingam[11] to propose that 5′-nucleotides are far more rigid than nucleosides, the conformation about the C4′–C5′ bond being *gg* whereas nucleosides exhibit all the three possible staggered conformations—*gg*, *gauche trans* (*gt*) and *trans-gauche* (*tg*). The only exceptions so far are 6-azauridine-5′-phosphate[12] and deoxyguanosine-5′-phosphate[13,14] which have *gt* conformation. The present studies show the general validity of Sundaralingam's proposal even in case of nucleoside (pyrimidine)-5′-diphosphates.

Pyrophosphate conformation

The pyrophosphate group both in CDP and CDP-choline has the characteristic staggered conformation. The bond lengths of the bridging O6′ to the two phosphorus atoms show significant differences: CDP: P1–O6′=1.58, P2′–O6′=1.62 Å, P1–O6′–P2=127.8°; CDP-choline: P1–O6′=1.60, P2–O6′=1 64 Å, P1–O6′–P2=131.7°. This suggests that the two halves of the diphosphate have different resonance hybrid states. The differences in the bond lengths are more pronounced than those found in the only other organic pyrophosphates so far solved[15–17], namely di-*β*-naphthyl pyrophosphate[15] (P1–O6′=1.59, P2–O6′=1.61 Å) and thiamine pyrophosphate[16] (P1–O6′=1.58, P2–O6′=1.58 Å). An interesting feature of the CDP and CDP-choline molecules is that while the pyrophosphates in both have the staggered geometry, their orientations in the molecules are strikingly different. The P–P vectors correspond to left and right rotations about the C5′–C4′ bond, the diphosphate group oriented towards the ribose hydroxyl oxygens in CDP and away from them in CDP-choline. It is as if the second phosphate in CDP-choline is linked with O11 as the bridging oxygen instead of O6′. These features are shown in Fig. 2.

CDP-choline molecule has a folded shape

The present analysis is the first one to give information about the conformation in the solid state of a nucleotide containing the pyrophosphate diester linkage. The geometry of this group and the choline moiety have features which lead to a highly folded shape. Figure 3 shows the molecules viewed down the P–P vector. The three fundamental angles O6′–P2–O7′–C7, P2–O7′–C7–C8 and O7′–C7–C8–N5 are 72.7, −166.7, and 68.1° and the choline moiety has thus the *trans* near-*gauche* conformation. This preferred energy conformer was found both in the crystal structures of choline derivatives[18,19] and in minimum potential energy computations[20] of the O–C–C–N^+ system. The only other crystal structures containing the phosphoryl-choline moiety which have been analysed are those of glyceryl phosphorylcholine[21] and its cadmium chloride complex[22], where similar conformations were found.

Interactions between molecules

Hydrogen bonding and other interactions between nucleotide bases, like and unlike, are fundamental to their recognition and function in biological systems. In the two structures reported here the cytosine base exists in two different tautomeric forms; protonated (CDP) and neutral (CDP-choline). Their interactions in the crystal structures are shown in Fig. 4.

As can be seen in CDP-choline, the imidine nitrogen N3 prefers, in the presence of the metal ion, to form an ionic bond rather than a hydrogen one. The ribose hydroxyl atoms also show a similar pattern. While they are involved in metal ligation in CDP-choline, in CDP they form strong hydrogen bonds with 2_1 screw hydroxyl oxygens. This hydrogen bonding network has a prominent role in the

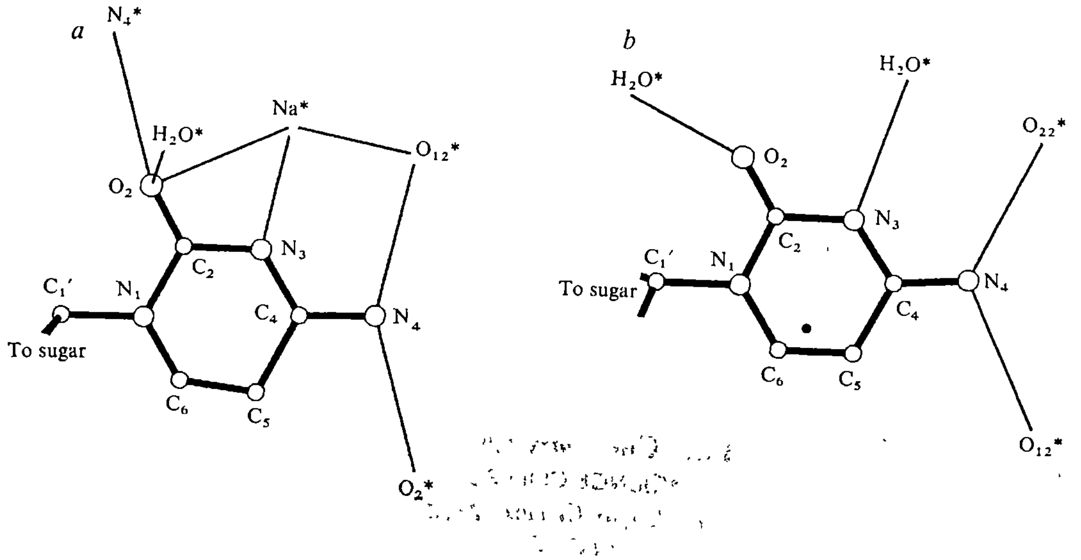

Fig. 4 Intermolecular interactions involving the base in: *a*, CDP-choline; *b*, CDP. An asterisk marks atoms related by crystallographic symmetry.

formation of the crystal lattice. The relationship between CDP-choline molecules in the extended crystal structure seems to be of particular interest.

The molecules related by a '*b*' cell translation are linked, base to the first phosphate moiety through Na atoms which also form strong ionic bonds with N3 and O11 (Na–N3= 2.39 Å, Na–O11=2.31 Å). These are then tightly linked with 2_1 screw related molecules both by metal ligation and hydrogen bonds involving O12 and both the ribose hydroxyl oxygen atoms. The result is that the CMP-5′ parts of the molecules are bound to each other rigidly in the structure while the phosphoryl choline groups are only loosely held with no such strong interactions with their environment. Their interactions with the neighbouring water molecules are much weaker as indicated by high temperature factors for the water oxygen atoms.

The incorporation of CDP-choline in the enzymatic synthesis of phospholipids like lecithin and sphingomyelin takes place with the transfer of the phosphoryl choline moiety from CDP-choline leaving CMP-5′ as the product

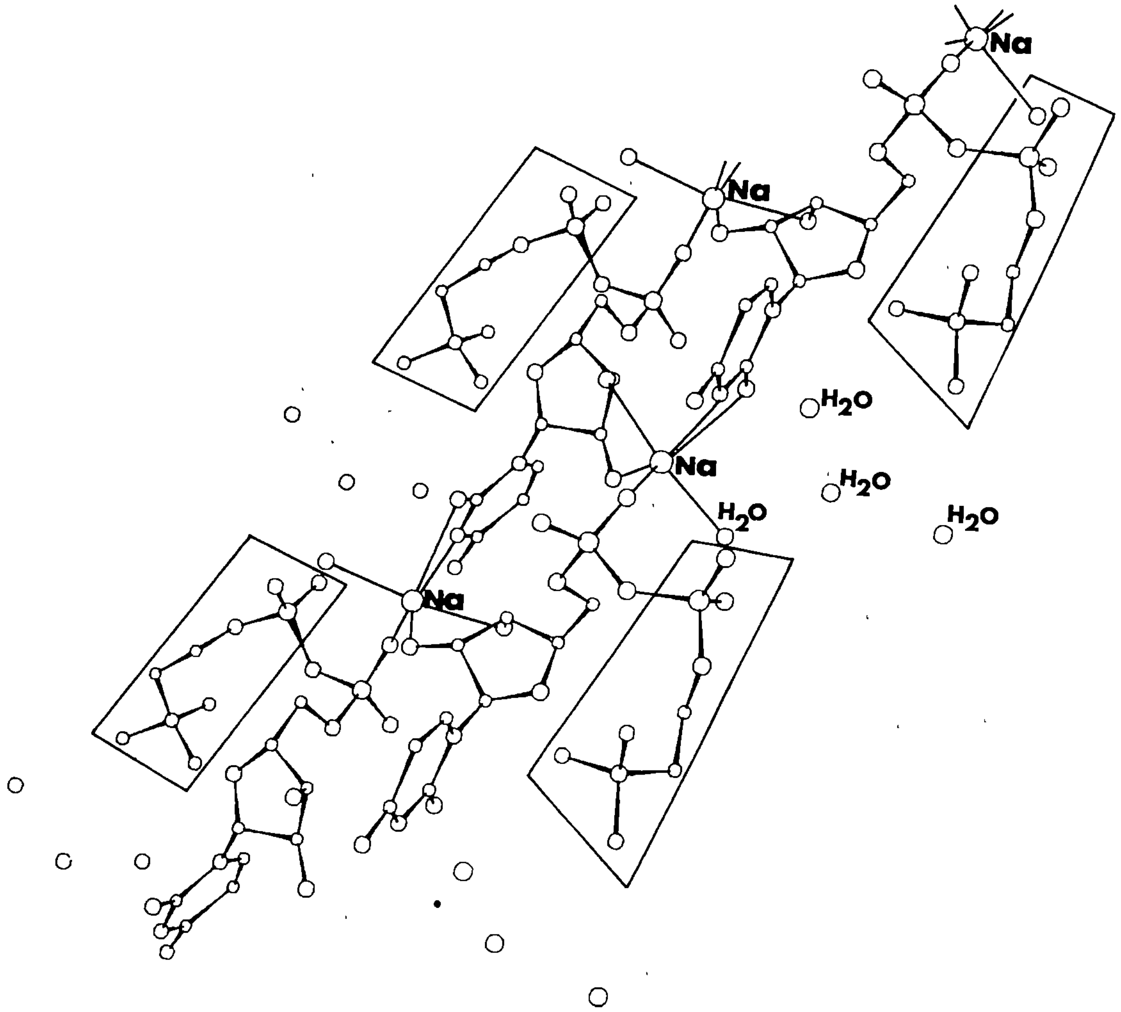

Fig. 5 Perspective packing view of CDP-choline and Na coordination. Phosphoryl-choline moieties are within boxes. Four molecules are shown.

of the reaction. On the assumption that enzyme recognition is influenced both by specific shape and substrate–substrate bonding, our experimental observations on the crystal structure of CDP-choline may well have relevance to the *in vivo* action of this coenzyme. The folded shape of the molecules with CMP-5′ parts strongly bound and the phosphoryl-choline groups only loosely held permits easy cleavage of the pyrophosphate bond facilitating the direction of such a reaction.

The present investigation is part of a collaborative project between the Indian Institute of Science, Bangalore and the University Chemical Laboratory, Cambridge. M.A.V. thanks the Indian MRC for financial support and the SRC (UK) for a Senior Visiting Fellowship. We also thank the MRC (UK) for financial support and the SRC for the provision of the diffractometer. O.K. is a member of the external staff of the MRC. M A.V. was on leave from the Indian Institute of Science.

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letters to nature

Nova in Cygnus of record brightness

THE recent bright nova in Cygnus is probably one of the most remarkable galactic novae observed. Invisible on the Palomar Sky Survey it had climbed through at least 18 mag to its maximum of $V = 1.8$ on August 31.0 (UT). This led to some early speculations whether this might be the first galactic supernova observed since Kepler's days. This suggestion is not, however, supported by the spectrum of the object and is also inconsistent with the extremely rapid decline in brightness after maximum. On the other hand, assuming it to be a classical nova, there are indications that it is a very luminous one.

Using the 61-cm Cassegrain–Nasmyth telescope of the Lund Observatory, we have obtained photoelectric *UBV* observations and a number of intermediate-dispersion spectrograms covering the time of maximum and the following week. A preliminary light curve is shown in Fig. 1. Our data have been supplemented with those of other observers. The light curve is smooth during maximum and early decline, but significant fluctuations appear during the transition stage; for example, a local maximum seems clearly established on September 7.88 UT.

For ordinary novae there is a statistical relation between absolute magnitude at maximum, M_0, and the time for a brightness decrease of three magnitudes from maximum, t_3. McLaughlin gives[1]

$$M_0 = -11.5 + 2.5 \log t_3$$

with t_3 expressed in d. For Nova Cygni we find $t_3 = 3.5$ d and $M_0 = -10.1$. The fastest non-recurrent nova hitherto known, CP Pup 1942, had $t_3 = 7$ d. For most novae, t_3 ranges from 10–200 d. Application of the above formula therefore depends on a somewhat hazardous extrapolation. It suggests, however, an unusually high luminosity. It should be noted that supernovae have values of t_3 around 50 d and absolute magnitudes from −15 to −20.

Nova Cygni is situated almost exactly in the galactic plane ($l^{\rm II} = 89.8°$, $b^{\rm II} = -0.1°$). The extinction in this direction is moderate. Assuming[2] an extinction of 2.5 mag for a distance modulus of 12, the nova would be ~ 800 pc away.

The photoelectric colours $U-B$ and $B-V$ indicated at premaximum a strong excess of ultraviolet and visual light compared with the blue region, approaching the colours expected for black-body radiation. At maximum brightness the colours were $U-B = -0.10$ and $B-V = 0.65$, decreasing gradually to −0.60 and 0.20 respectively 8 d after maximum. At this stage the colours mainly reflect the relative intensities of the emission lines.

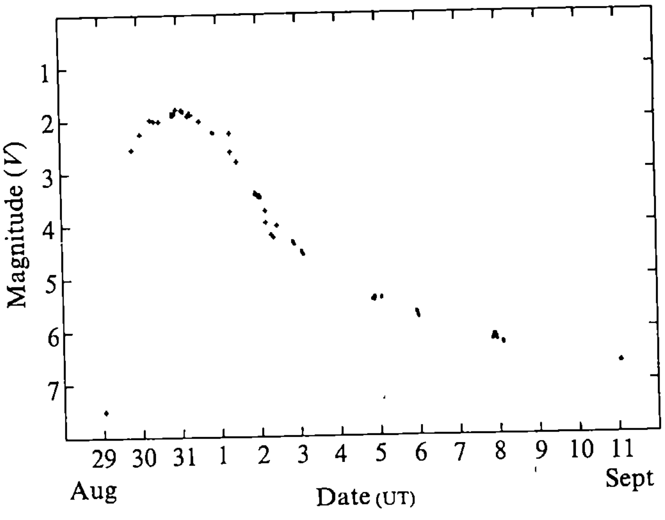

Fig. 1 Light curve of Nova Cygni 1975. Dots, our data, crosses, data from ref. 3

The spectral changes from one day before to five days after maximum are drastic. This is demonstrated in Fig. 2, which contains intensity registrations in the blue wavelength region. The evolution is roughly that of the typical nova spectrum, though it is unusually rapid and the narrow absorption lines normally seen seem to be missing. The premaximum spectrum is dominated by very broad, but not very strong, absorption lines of for instance H, Ca II, and Fe II, displaced by 1,000 to 2,000 km s^{-1} towards shorter wavelengths. At maximum, bright lines of the same elements appear close to their normal

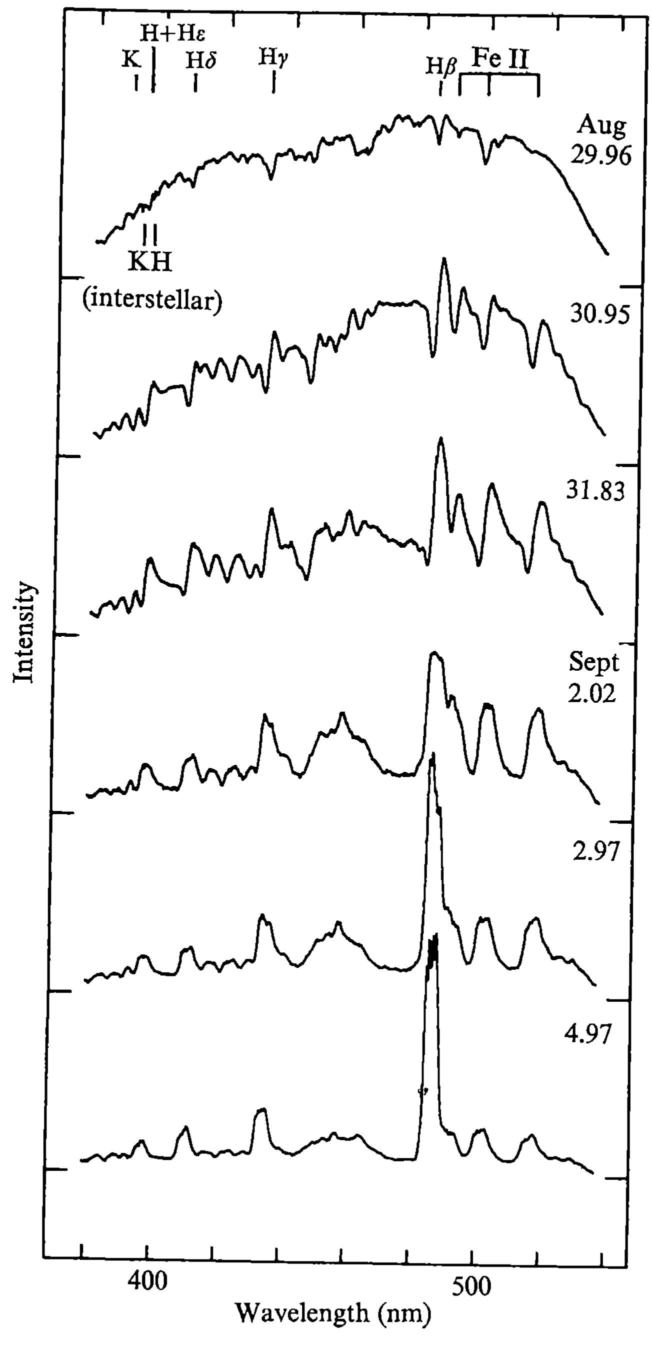

Fig. 2 Spectra of Nova Cygni. The zero-intensity point of each spectrum is indicated. Original dispersion was 83 Å mm^{-1} on IIIa-J plates.

positions. From then on the continuum weakens relative to the lines. A week after maximum most of the light comes from very broad, multiple-component emission lines. The structure of the lines changed markedly from night to night indicating that several clouds were ejected at velocities of about 1,500 km s^{-1}.

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The periodic transient X-ray sources

THE transient X-ray sources Ariel 1118−61 and Ariel 0535+26 have periods of 405 s (ref. 1) and 114 s (ref. 2) respectively. Rappaport and McClintock[3] reported the discovery of a 283-s period in the X-ray binary 3U0900−40 (a 'continuous' source) which is known to have an orbital period $P_{\rm orb} = 8.95$ d. This raises the possibility that the periodic transients may be a particular stage of evolution of the more familiar class of X-ray binary of the 3U0900−40 variety. We propose that the transient phenomenon occurs during the 'sleeping phase'[4] of the binary system when the early type star is still essentially on the main sequence, significantly underfilling its Roche lobe.

We assume that neutron stars are born rotating rapidly. Illarionov and Sunyaev[5] have shown that a magnetic, rotating neutron star will spin down in a stellar wind by a combination of the pulsar emission and 'propeller' mechanisms to an equilibrium period $P_{\rm eq}$ at which the Alfvèn radius is equal to the corotation radius. In general, significant accretion can occur only when $P \geqslant P_{\rm eq}$ where[5]

$$P_{\rm eq} \simeq 30\left[\frac{B_0}{10^{12}\ {\rm gauss}}\right]^{6/7}\left[\frac{1.5\times10^{-11}\dot{M}_\odot{\rm yr}^{-1}}{\dot{M}_{\rm A}}\right]^{3/7}\left[\frac{M_\odot}{M}\right]^{5/7}{\rm s} \quad (1)$$

Here B_0 is the strength of the surface magnetic field, M is the mass of the neutron star, and $\dot{M}_{\rm A}$ is the rate of accretion. For a neutron star accreting in a spherically symmetric wind of strength $\dot{M}_{\rm W}$ we also have

$$\frac{\dot{M}_{\rm A}}{\dot{M}_{\rm W}} \sim 10^{-4}\left[\frac{M}{M_\odot}\right]^2\left[\frac{20R_\odot}{a}\right]^2\left[\frac{10^3{\rm km\,s}^{-1}}{V}\right]^4 \quad (2)$$

where V is the velocity of the wind at the accretion radius and a is the binary separation. When accretion occurs the X-ray luminosity $L_{\rm X}$ is given by

$$L_{\rm X} \sim 0.1\,\dot{M}_{\rm A}c^2 \simeq 10^{35}\left[\frac{\dot{M}_{\rm A}}{1.5\times10^{-11}M_\odot{\rm yr}^{-1}}\right]{\rm erg\ s}^{-1} \quad (3)$$

For 3U0900−40, the mean X-ray luminosity observed at present ($\sim 10^{37}$ erg s^{-1}, assuming a distance of $\sim$2 kpc) indicates $\dot{M}_{\rm A} \sim 10^{-9}M_\odot$ yr^{-1}, which implies $P_{\rm eq} \sim$ 4–30 s for $B_0 \sim 10^{12}$–10^{13} gauss. For $V \sim$ 500–1,000 km s^{-1} the corresponding mass loss rate $\dot{M}_{\rm W} \sim 10^{-5}$–$10^{-6}M_\odot$ yr^{-1}. Unless we adopt what may be an unacceptably high value for B_0 ($\sim 10^{14}$ gauss), the 283-s periodicity in 3U0900−40, if interpreted as a rotation period of a neutron star, indicates that spin down has occurred in a weaker wind than is currently observed.

The time scales for spin down in the pulsar ($t_{\rm CR}$) and propeller ($t_{\rm A}$) phases have been discussed[5,6]. Assuming $B_0 \sim 10^{11}$ gauss, $\dot{M}_{\rm A} \sim 10^{-11}M_\odot$ yr^{-1} (equivalent to $\dot{M}_{\rm W} \sim 10^{-7}M_\odot$ yr^{-1}) Illarionov and Sunyaev[5] find that $t_{\rm CR} \sim 10^7$ yr and $t_{\rm A} \sim 10^8$ yr. They conclude that $t_{\rm A}$ is typically greater than the evolution time, $t_{\rm e}$, of an OB star ($t_{\rm e} \sim 3.7\times10^6\,(M_{\rm opt}/21M_\odot)^{-1}$[9]) (ref. 7) and that accretion is unlikely to occur during the main sequence phase.

Fabian[6] has discussed models for Ariel 1118−61 and shows that in a weak wind ($\dot{M}_{\rm W} \sim 10^{-10}M_\odot$ yr^{-1}) and with $B_0 \sim 10^{12}$ gauss, the spin down of a neutron star to periods of about a minute could occur in a timescale $\sim 10^8$ yr. He suggests that sporadic enhancements in the stellar wind (from $\sim 10^{-10}$ to $\sim 10^{-6}M_\odot$ yr^{-1}) could produce a transient source of luminosity $L_{\rm X} \sim 10^{36}$ erg s^{-1}.

In the particular case of 3U0900−40, however[8], $M_{\rm opt} > 20M_\odot$ indicating $t_{\rm e} < 5\times10^6$ yr. It would thus seem that spin down may be faster than previously supposed. Fabian[6] has drawn attention to uncertainties in the spin down estimates in the pulsar emission phase. Likewise details of spin down during the propeller phase are not well understood[5]. The estimated time scales could well be uncertain by an order of magnitude.

There is also the possibility that the stellar wind may vary with evolution (for example, observations of τ Sco (BOV) $\dot{M}_{\rm W} \sim 10^{-8} M_\odot$ yr^{-1} (ref. 9); ε Ori (BOIa) $\dot{M}_{\rm W} \sim 10^{-6} M_\odot$ yr^{-1} (ref. 10)). Using equations (9), (11) and (12) of ref. 5 with $\dot{M}_{\rm A} = 10^{-13} M_\odot$ yr^{-1} ($\dot{M}_{\rm W} \sim 10^{-9} M_\odot$ yr^{-1}) in the pulsar emission phase and $\dot{M}_{\rm A} = 10^{-11} M_\odot$ yr^{-1} ($\dot{M}_{\rm W} \sim 10^{-7} M_\odot$ yr^{-1}) in the propeller phase, we find for $B_0 \sim 10^{12}$–10^{13} gauss, $t_{\rm CR} \sim 10^7$–10^6 yr, $t_{\rm A} \sim 10^7$–3×10^6 yr and $P_{\rm eq} \sim 30$–300 s. Thus a weaker wind during the pulsar phase could reduce the overall spin down time by allowing the more efficient pulsar emission mechanism to operate for longer before the propeller takes over. Because of free–free absorption in the wind it is unlikely that the pulsar would be detectable at radio energies during spin down[5].

Based on the above considerations we propose that spin down of the neutron star to periods of about a minute occurs while the OB star is still essentially on the main sequence. Van den Heuvel[4] has described this phase of evolution of the pre-X-ray binary system following the formation of a neutron star, as 'the sleeping phase'. We suggest that the newly born X-ray source ($P_{\rm eq} \sim$ minutes) may be detected during this phase as a periodic transient (for example, during an outburst) or as a weak ($L_{\rm X} \sim 10^{35}$ erg s^{-1}) X-ray source. The transient nature could be attributed to any one of many possibilities: (1) the tendency of the accreting neutron star to spin up in the wind and temporarily inhibit accretion. This mechanism would only be effective when the rotation period of the neutron star is near the 'equilibrium' value appropriate to the stellar wind strength (for example, Cen X-3 during the present epoch, and possibly 3U0900−40 during its main sequence phase). (2) The Buff and McCray[11] thermal instability which may manifest itself in a weak wind situation. (3) Matter build up near the Alfvèn radius and impulsive accretion towards the end of the propeller phase. (4) Sporadic variations in the wind strength by a factor ~ 10.

When accretion does occur the neutron star may spin up. For 3U0900−40, the estimates[6] indicate a current spin up rate of 10^{-2}–10^{-1} s yr^{-1} for $V \gtrsim 500$–$1{,}000$ km s^{-1}. Since the intense wind phase ($\dot{M}_{\rm W} \sim 10^{-5}$–$10^{-6} M_\odot$ yr^{-1}) lasts for ~ 10^4 yr (ref. 12) until Roche lobe overflow occurs, 3U0900−40 is not expected to have spun up appreciably since its main sequence phase. When Roche lobe overflow occurs the X-ray source will probably be smothered. It is interesting to note that the optical stars of the nearly continuous X-ray sources appear to be near their Roche lobes[13].

It is not clear whether systems like Cen X-3 and 3U1700−37 which are of short orbital period with very early type stars and strong stellar winds, would follow the evolution described above. In such systems the optical star essentially reaches its Roche lobe during hydrogen core burning and the postulated weak wind phase may be short lived. (The Roche lobe radii of Cen X-3 and 3U0900−40 are ~ $12 R_\odot$ and ~ $35 R_\odot$ respectively.) Estimates similar to those made for 3U0900−40 indicate that Cen X-3 is unlikely to have spun down to periods $P \gtrsim 40$ s. Indeed for Cen X-3 the currently observed X-ray luminosity (~ 5×10^{37} erg s^{-1}) yields $P_{\rm eq} \sim 2$–15 s for $B_0 \sim 10^{12}$–10^{13} gauss, consistent with the observed 4.8-s period[14]. This could be a system in which the rotation of the neutron star has kept up with the wind evolution. By analogy 3U1700−37 may be expected to have a short period ($\lesssim$ 1 min).

In the above model the optical counterparts of periodic transients are OB stars, which are relatively unevolved and underfill their Roche lobes, in orbit about the transient with periods $P_{\rm orb} \lesssim 20$ d. Since the 'sleeping phase' lasts typically a hundred times as long as the intense stellar wind phase[4] we may expect to see a hundred times as many transients as nearly continuous X-ray sources, of the 3U0900−40 variety. Since the transients are expected to be intrinsically less luminous, however, they may be detectable only during a flare.

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de Sitter's theory 'melts' Europa's polar cap

DURING the favourable passage of the Earth through the plane of Jupiter's equator in 1973 many observations of the mutual phenomena of the Galilean satellites were obtained. Since then there have been numerous preliminary analyses[1,2] arguing for the presence of a bright polar cap on Europa (J2), based essentially on photoelectric observations of a series of occultations of J2 by J1 (Io) in which increasingly larger fractions of the north polar region of the former were covered at mid-event with each successive occultation. These events—and especially the near-grazing, or polar ones—were substantially deeper than predicted. An inevitable explanation, in view of the finding of ice[3] on J2 and J3, was the suggestion of a bright north polar cap for J2.

Alternatively, it may be that at mid-event the projected minimum distance, z, between the centres of the satellites in a direction essentially parallel to Jupiter's polar axis, was smaller than predicted by Sampson's theory[4,5] (which has been in general use since 1915 to compute the ephemerides of the Galilean satellites). This explanation at first seemed less likely for several reasons. Although recent timings of eclipses in Jupiter's shadows have shown, as have the mutual events, that the theory now suffers from considerable errors of longitude, the latitude errors (that translate directly into errors, Δz, in z) were believed to be much smaller. Furthermore, preliminary analyses of a few occultations of J2 by J1 suggested that to remove the need for a bright polar cap on J2 required Δz corrections too large to be compatible with the observed durations of the occultations.

Contrary to these early findings, we can show that the 'Δz explanation' is indeed the more plausible one. We have analysed some 30 occultations and 10 eclipses, from which we have selected 10 well-observed and characteristic light curves listed in Table 1, where 'O' and 'E' in column 1 stand for 'occults' and 'eclipses', respectively. Column 2 gives the observed mid-time of an event whose amplitude, ΔV, in visual magnitude, is in column 3. We have fitted a theoretical curve to each observed one by making least-squares adjustments to the longitudes of the satellites and to the parameters, z, R_2, and β. Here, R_2 denotes the radius of J2 (that of J1 is well

Table 1 Data from analyses of light curves

Event	Mid-time 1973 (UT)	ΔV	de Sitter–Sampson Δz_1	Δz_2	$\Delta z_1-\Delta z_2$*	Solution 1 Δz*	Δ_1	Solution 2 β	Δ_2	Δ_0
102	June 10.38194	0.09	86	462	−376	−426	0.0034	2.88	0.0140	0.0031
102	June 17.46936	0.17	101	455	−354	−415	0.0049	1.79	0.0115	0.0039
102	June 24.55613	0.24	116	447	−331	−343	0.0032	1.42	0.0073	0.0060
102	July 22.89868	0.43	166	409	−243	−268	0.0070	1.15	0.0079	0.0054
102	Aug. 16.70257	0.53	207	375	−168	−187	0.0043	1.07	0.0051	0.0046
1E2	Aug. 16.73448	1.53	228	399	−171	−185	0.0088	1	0.0261	0.0098
102	Sept. 10.53082	0.62	253	357	−104	−116	0.0056	1.01	0.0058	0.0055
1E2	Sept. 10.61979	1.40	284	423	−139	− 82	0.0120	1	0.0161	0.0161
102	Sept. 28.30912	0.55	286	378	− 92	−112	0.0043	0.96	0.0050	0.0050
201	Dec. 21.02251	0.15	−124	−539	+415	+425	0.0076	1.85	0.0090	0.0056

*A positive Δz or $\Delta z_1-\Delta z_2$ implies an increase in the projected centre–centre distance z if J1 is north of J2 (first 7 events), but a decrease if J1 is south of J2 (last 3 events).

known from its occultation of β Sco[6]) and β is, for the occulted satellite, the ratio of the average surface brightness of the occulted portion at mid-event to the mean over the entire disk. A consideration of all the total and near-total occultations and eclipses of J2 enabled us to obtain R_2 = 1,533 km, a downward revision of only 17 km from the micrometric value[7]. Table 1 shows the results of two solutions to the observed curves, both of which use R_2 = 1,533 km. The first, or 'Δz solution' in columns 7 and 8, sets $\beta = 1$ and obtains Δz; the second, or 'polar cap solution' (columns 9 and 10) imposes $\Delta z = 0$ and yields β. (The longitude corrections are identical for both solutions, but are not relevant here. We also omit a third, simultaneous solution for Δz and β, resulting in β values not much different from 1.) Also shown in Table 1 are the r.m.s. residuals, Δ_1 and Δ_2, for the two solutions, where the intensity scale was normalised to unity outside of the events. A third set of residuals, Δ_0, was obtained independently of any of a model light-curve by folding the two branches of each light curve. Since these curves give no indication of asymmetry, Δ_0 provide a measure of the noise levels.

Table 2 Elements for J1 and J2 (t (d) from 1900 Jan. 0.5 ET)

Author	γ_1	θ_1	l_1
Sampson	0.02725°	33.299°−0.134068° t	142.59987°+203.488954208° t
de Sitter	0.0317°	64.7° −0.13214° t	142.590° +203.48895460° t
	γ_2	θ_2	l_2
Sampson	0.46805°	290.54986°−0.0327375° t	99.55081°+101.374723445° t
de Sitter	0.4668°	292.81° −0.032599° t	99.530° +101.37472360° t

Those residuals, especially for the shallow occultations, clearly favour solution 1. The β for these shallow events correspond, when one recalls that the geometric albedo of J2 lies near 0.6 (ref. 8), to an unrealistically high surface brightness for the north polar region of the satellite. Occultations 2 and 3 are the ones that were used qualitatively to support the polar cap[1]. In the case of the final entry of Table 1, the north pole of J1 was covered by J2. This event requires β = 1.85 if Δz is forced to be zero, and yet photography from Pioneer 10 has shown that this region of J1 is slightly darker than the average surface material.

Table 1 also includes two eclipses, for which Δz agrees with that of the occultations which occurred just beforehand. While the radius R_2, derived from the occultations, changed little with Δz, R_2 obtained from the eclipses gave the discordant values 1,345 and 1,610 km when Δz was forced to be zero. When Δz was included in the solution, these values became 1,543 and 1,519 km. (For eclipses, β was set equal to unity. To have set β free would have involved a substantial, and apparently unnecessary, complication because penumbral shadowing must be considered.)

Perhaps the strongest evidence for the reality of the Δz corrections comes from an independent source: de Sitter's theory[9,10] for the Galilean satellites. Table 2 lists the proper inclinations, γ_1 and γ_2, on a plane essentially coincident with Jupiter's equator; the proper nodes, θ_1 and θ_2; and the mean longitudes, l_1 and l_2, as obtained by Sampson and by de Sitter for J1 and J2. Whereas the mean longitudes and inclinations generally agree, the nodes differ markedly, especially when brought forward to 1973. A strong case exists for favouring de Sitter's values because his reliance on heliometer and photographic measurements—in addition to eclipse observations—provided a better determination of the orbital planes of J1 to J4.

The small differences in the two determinations of γ_1 and γ_2 yield latitude discrepancies of no more than 33 km for J1 and 15 km for J2. The marked differences, however, in the positions and motions of θ_1 and θ_2 between the de Sitter (D) and Sampson (S) values have a much larger influence. The effect of the differing values for the inclinations and the arguments of latitude, corrected for light travel time to Jupiter, on Δz can be calculated at the mid-times of the events from Table 2 and the formula

$$\Delta z_i = a_i[\gamma_{i,D}\sin(l_i-\theta_i)_D-\gamma_{i,S}\sin(l_i-\theta_i)_S], \quad i = 1, 2$$

where a_i is the semimajor axis of satellite i. The differences, $\Delta z_1-\Delta z_2$ (km), in Table 1 agree very well with the Δz corrections derived from the light curves. There is some indication that $|\Delta z| > |\Delta z_1-\Delta z_2|$, particularly for the shallow, 102 cases. In view of photometric uncertainties, we regard this as of marginal significance. If real, it would mean a slight revision of de Sitter's orbital elements and/or a minute polar brightening. The general agreement (shown in Table 1) seems to lead to a ready explanation for the behaviour of the J1–J2 events in 1973 and to add confirmation to de Sitter's theory. We are now completing the theory so that calculations of ephemerides can be made.

Whether any information on the brightness of satellite features can be obtained from these observations has yet to be shown.

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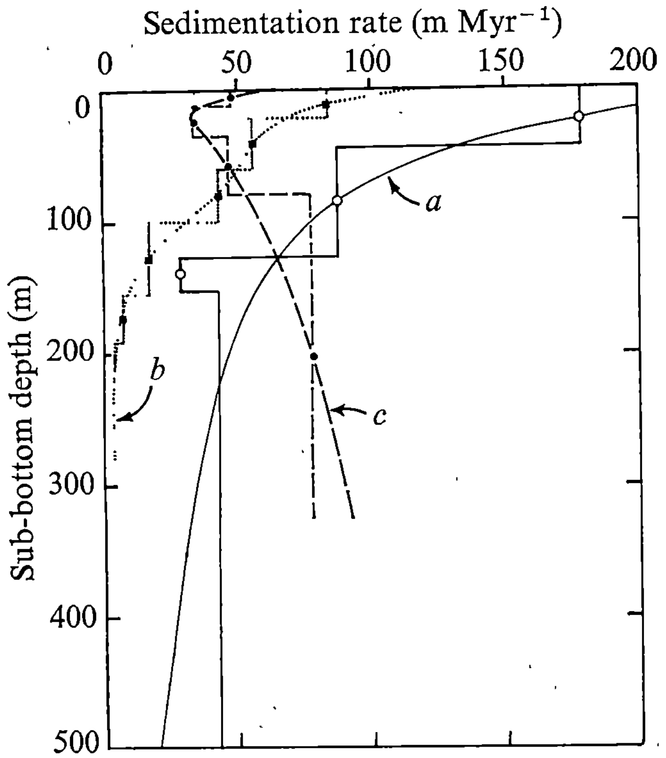
Late Cainozoic explosive eruptions in the Aleutian and Kuril Island Arcs

THE rate at which major explosive eruptions have occurred in the Aleutian and Kuril Island Arcs is recorded in tephra layers recovered in cores of the Deep Sea Drilling Project (DSDP). Major tephra eruptions occurring with the frequency and intensity currently observed in the Aleutian and Kuril Arcs may have marked effects on world climate, especially through the production of dust veils and the consequent variation in the volume of ice in the polar seas and in general atmospheric circulation[1].

Since 1760 the 36 active volcanoes in the Aleutian Island Arc have erupted 154 times[2]. Of these, 32 were 'major explosive eruptions'[2]. On the Kamchatka Peninsula, 23 active volcanoes of the Kuril Island Arc have erupted at least 137 times since 1729 (ref. 3). Several of these eruptions were sufficiently energetic and voluminous to blast tephra into the stratosphere and to blanket extensive areas with pyroclastic debris. The 1912 eruption of Mount Katmai in Alaska deposited 25 cm of ash 160 km to the south-east, at Kodiak Village[4], and 7 cm of ash fell 560 km from the mountain[5]. The 1956 eruption of Bezymianny Volcano on Kamchatka produced an ashcloud 45 km high and deposited ash over 40,000 sq km (ref. 6). The 1964 blast at Shiveluch Volcano on Kamchatka deposited 2.5 mm of ash on the Komandorsky Islands, 400 km to the east[7]. The violent eruption of Mount Spurr, Alaska in 1953 shot a mushroom cloud upwards to a height of 21 km in about 40 min (ref. 8).

Fig. 1 Sedimentation rate against sub-bottom depth curves for DSDP sites 178 (*a*), 183 (*b*) and 192 (*c*) in the northern Pacific ocean. Palaeontological data from refs 9 and 10.



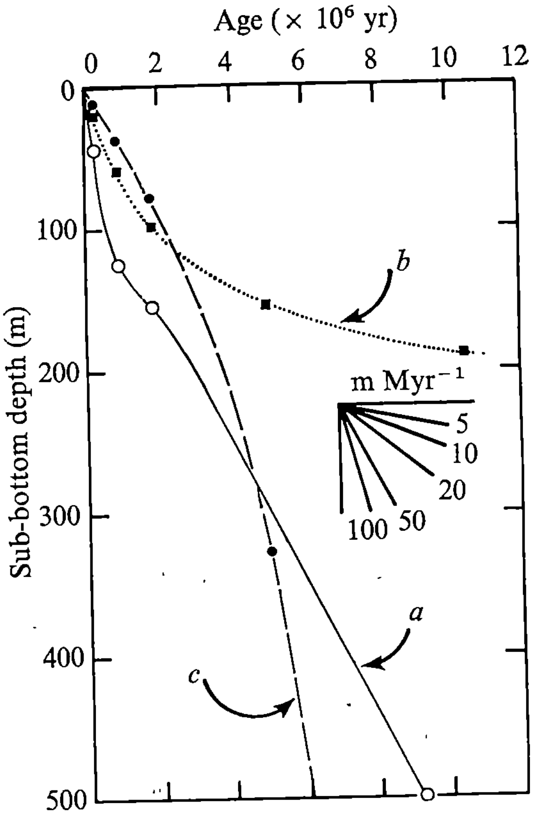


Fig. 2 Age against sub-bottom depth curves for DSDP sites 178 (*a*), 183 (*b*) and 192 (*c*). Data and age assignments as in Fig. 1.

DSDP sites 178 and 183 are in the Gulf of Alaska, 450 and 250 km, respectively, south-east of the Aleutian volcanic chain[9,10]. Site 192 lies 350 km east of the nearest volcanoes in the Kuril-Kamchatka chain[10]. All three lie downwind from active volcanic centres. Cores recovered at the sites contain large quantities of volcanic ash, particularly in the Pleistocene sections.

The rate of ash fall at each site has been estimated by using each recovered interval as a sample of the rate at which tephra was deposited at each site. The time span represented by each recovered interval has been estimated by dividing the sedimentation rate by the length of the recovered interval. The sedimentation rate was obtained from an enlargement of the sedimentation rate against the sub-bottom depth curve (Fig. 1). The number of ash layers occurring within the time span represented by each recovered interval has been normalised to an arbitrary deposition rate of tephra layers each 1 Myr. The absolute age for the midpoint of each recovered interval has been estimated from the sub-bottom depth against the age curve (Fig. 2) and plotted against the rate of tephra arrival (Fig. 3).

This technique of using each recovered interval as a 'sample' of the rate of incoming tephra is required because core recovery is very low. At site 178 only 39% of the tephra-rich interval was recovered, at site 183 only 20%,

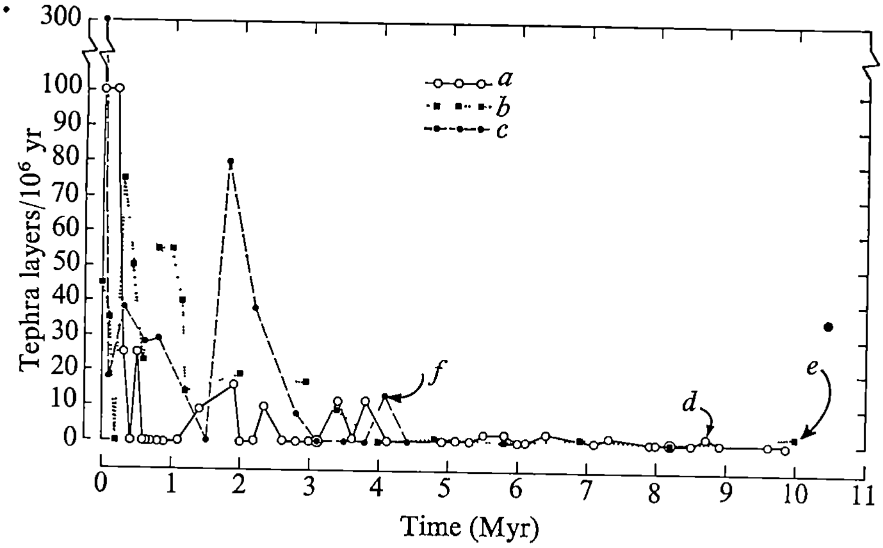


Fig. 3 Rate of ashfall in the northern Pacific ocean at DSDP sites 178 (*a*), 183 (*b*) and 192 (*c*). *d*, Oldest ash, site 178; *e*, oldest ash, site 183; *f*, oldest ash, site 192.

and at site 192 only 30%. Therefore, the actual number of ash layers is rather insignificant, and the rate of ash accumulation is a more appropriate parameter for analysis.

Tephra erupted in quantities sufficient to form distinct layers apparently began falling in the Gulf of Alaska about 10 Myr ago—but at a rate which averaged less than 1 layer every Myr—until about 4 Myr ago. Both curves from the Gulf of Alaska sites show a sporadic increase of up to 20 layers Myr^{-1} between 4 Myr and about 1.2 Myr BP, a decrease to 0 at 0.2–0.4 Myr BP, and a dramatic increase to rates of about 100 layers each Myr at present.

In the north-western Pacific, ash does not appear as discrete layers in the cores until about 4 Myr BP. It appears again at about 1.8 and 0.3 Myr BP, with an increase to 300 layers Myr^{-1} at present.

Summing up the data for sites 178 and 192 suggests that tephra layers are accumulating in the far northern Pacific at a rate of about 1 layer every 2,500 yr. This rate in itself is probably not indicative of any major influence on the Earth's atmosphere, for volcanic outbursts and associated effects are of short duration[1]. But because the layers in the cores are usually several centimetres thick and accumulated hundreds of kilometres from possible sources, the data probably only reflect major cataclysmic eruptions, and may only indicate a quickening pace or tempo of volcanism.

A survey of explosive volcanism over the past two centuries indicates there have been two primary latitudinal source belts of volcanic ejecta: a narrow belt just below the Arctic Circle, and a broad belt centred on the Equator[11]. In the Arctic Circle belt the height of the tropopause is only two-thirds of that over the Equator. A less explosive event in the northern latitudes could contribute as much or more debris to the northern stratosphere as an eruption on the Equator of the magnitude of Agung[11].

Data on the number of volcanic ash layers sampled at nany DSDP sites indicate an apparent global increase in Quaternary explosive volcanism[12]. A comparison of the ishfall rate curve for the northern Pacific sites with volcanic debris accumulation rates from the western Pacific[13], the Caribbean Sea[14], and other volcanic regions[12], suggests that most island arcs are experiencing comparable rates of cataclysmic eruption. The total world rate may possibly be 5–10 times that in the northern Pacific, which would suggest a major eruption every 250–500 yr. Ash layers preserved n the Antarctic ice sheet suggest even higher figures with nfalls during the peak period of volcanic activity (20,000–6,000 yr BP) reaching 30–40 a century[15]. Those rates, coupled with the apparently prodigious explosive eruptive history not recorded in the deep sea, could certainly have asting and significant effects on the albedo and air temperature of the Earth.

The magnitude of any effect is difficult to estimate directly, but it is known that dust injected into the stratosphere at high latitudes seems to tend to spread as a veil between the pole and about 30° latitude in either hemisphere[1]. Volcanic dust may cause an overall decrease in the amount of solar radiation received and change the meridional gradient of the insolation, and thus the north–south temperature pattern which governs the speed and pattern of the general circulation[1]. Depletion of the incoming solar beam is greatest at high latitudes, and depression of surface temperatures is probably also greatest at high latitudes[1]. The 1912 Katmai eruption was followed by a 10% increase in the total extent of sea ice at the end of the melting season—an effect which lingered through seven successive summers[16]. On a longer time scale there does seem to be some evidence that the late Wisconsin and Neoglacial ice advances were contemporaneous with major phases of volcanic activity[15,17] and the greatly increased worldwide level of volcanism[12] closely coincides with the major development of the late Cainozoic glacial ages.

A complicating factor affecting these calculations is the north-westward motion of the Pacific Plate relative to the North American and Asian Plates. Using a figure of 6×10^{-7} degrees yr^{-1} for the rate of motion between those plates, Ness and Kulm[18] calculated about 220 km of north-westward motion for site 178 during the past 5 Myr. Similar movements probably affected sites 183 and 192, for all the sites lie on the same plate. As a result of this motion, at least some of the increased rate of ashfall may be attributable to the DSDP sites in the northern Pacific simply moving nearer the volcanic sources. This may explain why evidence is not seen in the cores for the middle Miocene episode of calc-alkaline magmatism reported from the Aleutian Island Arc[19].

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Antarctic glaciation and early Tertiary vegetation

PALYNOLOGICAL evidence obtained from drilling in the Ross Sea by the Glomar Challenger suggests that vegetation persisted in the area until the late Oligocene and results from the same drilling programme also suggest that ice-rafting of clastic debris commenced in the late Oligocene[1]. The vegetation reflected in the pollen spectrum therefore seems to have been contemporaneous with early phases of glaciation.

Pollen and spores were recovered from site 270, which was drilled off the edge of the present Ross Ice Shelf (Fig. 1). The sequence at site 270 is shown in Fig. 2. Sediments of probable preglacial origin at the site are thin, consisting of a unit of quartz sand 1 m thick, lying on basal breccias and gneisses, and a further metre of glauconitic sandstone. Overlying these is a thick, pebbly, silty claystone of glacial marine origin. Subunits can be identified within this thick unit on the basis of the presence or absence of stratification. The preglacial glauconitic sandstone has been dated at 26±0 4 Myr using the K–Ar method[2], an age with accords with that suggested by rare dinoflagellates in the unit[3]. The change from preglacial to glacial marine conditions occurred over a short time interval, since the lower subunits of the glacial marine sequence have also been dated as Oligocene on the basis of their contained foraminifera[4].

Samples of preglacial and glacial marine units were macerated for pollen and spores. The preglacial units were barren of these microfossils, but all of the samples from the glacial marine unit were productive, though to a varying degree. The yield was highest in the basal subunit, 2J (Fig. 2), a laminated, burrowed siltstone with rare dropped pebbles. Degraded tissues, leaf cuticles and vascular fragments were also common in this subunit, suggesting deposition at no great distance from a vegetation source.

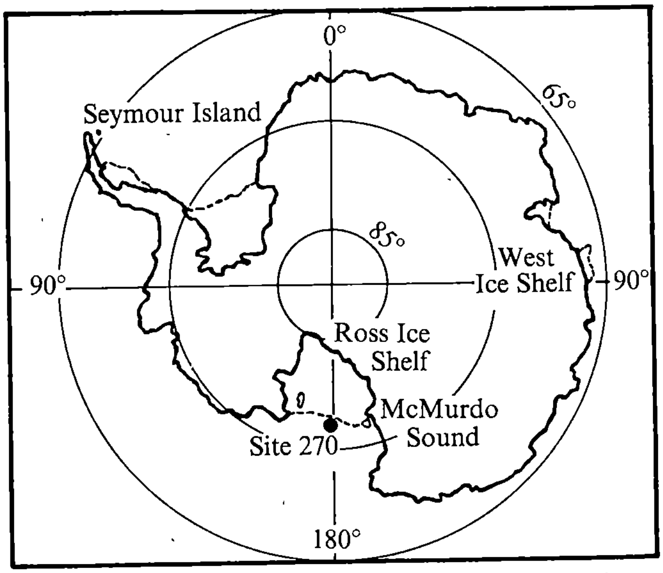

Fig. 1 Map showing Antarctic localities from which Tertiary plant microfossils have been recovered.

As in all glacial sequences, it is difficult to ascertain whether the plant microfossils in subunit 2J were derived from a parent vegetation which flourished during deposition of the unit, or whether they are recycled from older deposits. Modern glacial marine sediments on the Antarctic continental shelf contain recycled pollen and spores ranging in age from Palaeozoic to early Tertiary[5–7]. But in the assemblages from subunit 2J there are no forms which are obviously recycled; there is none derived from Palaeozoic and Mesozoic sequences in the Transantarctic Mountains, nor any of the dinoflagellate cysts which are abundant in the Black Island and Minna Bluff erratics. All of these older forms do, however, occur as a clearly recycled element in the upper parts of the glacial marine sequence at site 270. Their absence from the basal section suggests that little recycling occurred during deposition of the lower subunits, and that the pollen present reflects a parent vegetation that coexisted with the earliest glaciers.

Form species identified in subunit 2J, and their probable

Table 1 Form species of spores and pollen recovered from subunit 2J, site 270, and their probable botanical affinities

Pollen	Affinity
Podocarpidites spp.	Podocarpaceae
Lygistepollenites florinii (Cookson and Pike) Stover and Evans	Podocarpaceae (cf. *Dacrydium* spp.)
Phyllocladidites mawsonii Cookson	Podocarpaceae (cf. *Dacrydium franklinii*)
Microcachryidites antarcticus Cookson	Podocarpaceae (cf. *Microcachrys*)
Podosporites microsaccatus (Couper) Dettman	Podocarpaceae
Nothofagidites heterus (Cookson) Stover and Evans	*Nothofagus* (*brassi* group)
N. matauraensis Couper	*Nothofagus* (*brassi* group)
N. vansteenisi (Cookson) Stover and Evans	*Nothofagus* (*brassi* group)
N. flemingi (Couper) Potonie	*Nothofagus* (*fusca* group)
N. cf. *asperus* (Cookson) Stover and Evans	*Nothofagus* (*menziesii* group)
Tricolpites cf *matauraensis* Couper	Unknown
T. cf. *fissilis* Couper	Unknown
Triporopollenites sp.	Unknown
Proteacidites minimus Couper	Proteaceae
P subscabratus Couper	Proteaceae
P cf. *pseudomoides* Stover	Proteaceae
P. spp.	Proteaceae
Myrtaceidites spp.	Myrtaceae
Spores	
Cyathidites spp.	Cyatheaceae
Gleicheniidites cerciniidites (Cookson) Dettman	Gleicheniaceae
Laevigatosporites major (Cookson) Krutzsch	Unknown
Polypodiisporites sp.	Polypodiaceae
Lycopodiumsporites sp.	Lycopodiaceae
Stereisporites antiquasporites (Wilson and Webster) Dettman	Sphagnaceae

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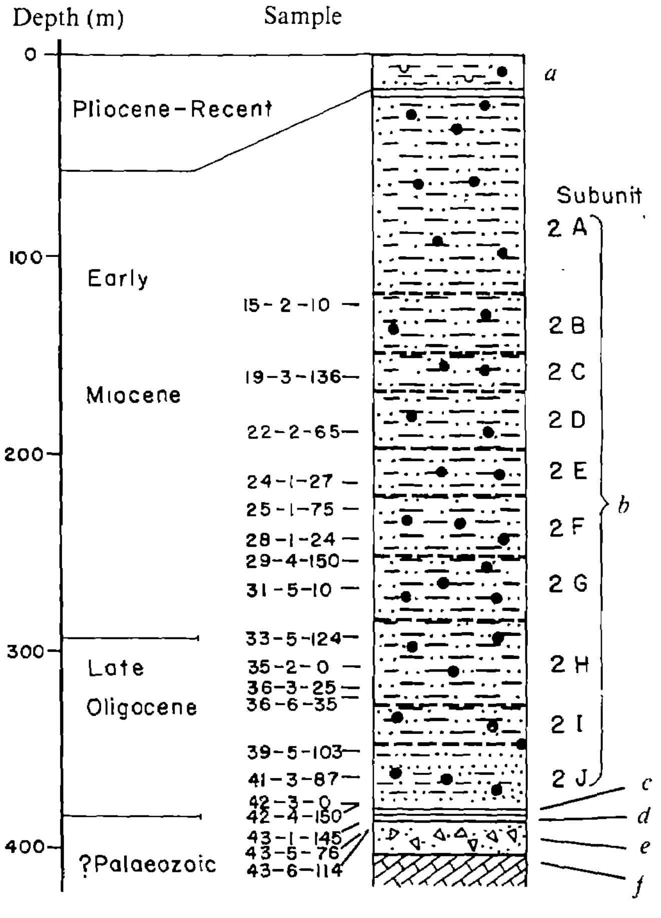

Fig. 2 Lithological sequence at site 270. Lithological subdivisions are shown to the right of the column; positions of cores from which palynological residues have been recovered are shown on the left. *a*, Unit 1: diatomaceous silty clay with pebbles; *b*, unit 2: silty claystone with pebbles (glacial marine); *c*, unit 3: greensand; *d*, unit 4: quartz sand; *e*, unit 5: breccia; *f*, calc-silicate gneiss.

botanical affinities, are listed in Table 1. Numerically, the assemblages are dominated by pollens of the *Nothofagus* type, with fossil forms similar to pollen of the extant *fusca* group present in slightly greater abundance than those of the *brassi* group. Pollen of Proteaceae is also well represented, but not diverse, with small, smooth walled pollens referable to *Proteacidites minimus* being most common. Small myrtaceous pollens, not identifiable below family level, occur rarely, as do tricolpate and triporate pollens that could be derived from a variety of parent families. Among gymnosperm pollens, podocarpaceous types predominate. the long ranging *Microcachryidites antarcticus* and *Podosporites microsaccatus* are most common. *Lygistepollenites florinii*, of mid-Palaeocene to Miocene range in south-eastern Australia[8], is a rare component. Cycadophyte pollens were not identified. Fern spores are rare, and chiefly suggest Cyathaceae, but include rare polypodiaceous types.

The assemblage is much less diverse than those of early Tertiary sequences in Australia and New Zealand. It is also less diverse than the Seymour Island fossil assemblages. It is almost identical with that from the Black Island erratics, which, on dinoflagellate evidence, is probably late Eocene. The microflora from the erratics has been interpreted as reflecting Southern Beech forest of the kind at present growing in cool temperate South America and New Zealand[9]. Temperatures cooler than those of Eocene New Zealand are, however, suggested, as pollen types indicative of warmth in the New Zealand Eocene are absent.

The data presented here reflect a late Oligocene parent vegetation in the Ross Sea region which was essentially similar to that of the erratics. A *Nothofagus*-dominated vegetation, with subordinate podocarps, Proteaceae and Myrtaceae, seems to be represented by the pollen spectrum. The available record suggests that little change in floristic composition occurred with the increasing deterioration of the Antarctic climate from the Eocene through to the Oligocene. There is no evidence of a vegetation phase dominated by herbaceous elements; taxa which are important in the modern sub-Antarctic vegetation, such as Graminae, Cyperaceae, Compositae and Umbelliferae have not been identified. It may be that those groups, which, on a global basis, achieved their major radiation only in the Neogene, were prevented from becoming established in the Ross Sea region by the onset of conditions limiting growth there early in that interval. Alternatively, this absence may only reflect the inadequacies of the fossil record.

The relationship between vegetation cover and the extent of glaciation in Antarctica in the early Cainozoic remains problematical. The persistence of some form of vegetation around the Ross Sea until at least the late Oligocene is not at odds with geological evidence bearing on the earliest stages of glaciation. Sedimentation rates have been used in this context to give a broad guide to the extent of ice cover[10]. High rates of sedimentation are believed to reflect an environment with wet base, 'temperate' glaciers calving bergs into a relatively open sea; by contrast, periods of intense cold, with the major development of ice shelves are distinguished by slow sedimentation and erosion by currents beneath the ice. High sedimentation rates (41 m Myr^{-1}) prevailed through the Oligocene and early Miocene sequence at site 270[11]. This, and the observation that pebbles in this part of the sequence are derived from inland Marie Byrd Land, suggest that the Ross Ice Shelf was not in existence during that interval, and that glaciers from Marie Byrd Land calved directly into an open Ross Sea embayment. Such a situation seems compatible with limited vegetation cover, which was perhaps confined to coastal zones and interglacier elevations.

It is not possible to determine precisely when adverse environmental conditions eliminated the Ross Sea vegetation entirely. The Miocene part of the drilled sequences in the region yields pollen similar in composition to that of the Oligocene and Eocene, but this occurs in very low frequencies only, and there is evidence of extensive reworking from older deposits. It seems likely, however, that the almost complete elimination of vegetation occurred sometime during the Miocene; this event probably occurred appreciably before the major ice advances of the early Pliocene[1].

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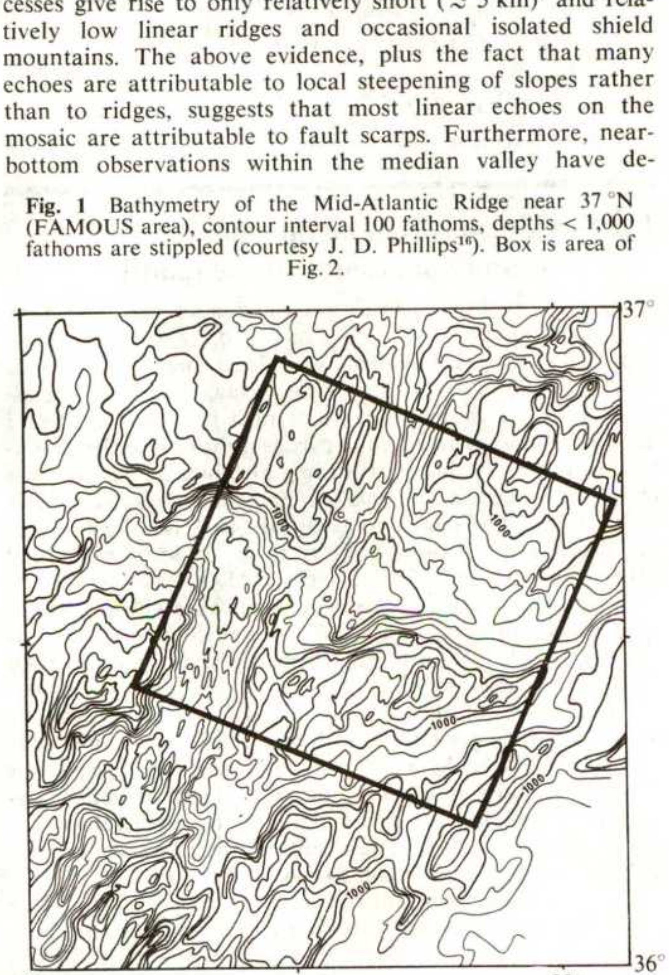
The fault pattern of a slow-spreading ridge near a fracture zone

In July, 1973 a 30,000 km^2 area of the median valley and crest of the Mid-Atlantic Ridge near 37°N (Fig. 1) was surveyed with the long range side-scan sonar, GLORIA[1]. A mosaic of overlapping sonographs was produced, part of which is shown in Fig. 2, representing the view of the sea floor which would be obtained if the water were removed and the sea bed illuminated from the north-west at a Sun elevation of about 15°. On the mosaic, white areas represent relatively strong backscattering targets, and the black areas are either in shadow or are weakly backscattering sediment ponds.

On the mosaic, numerous sub-parallel linear echoes, oriented at about 020°, and therefore parallel to the median valley, can be followed continuously for tens of kilometres. Narrow beam echo-sounder profiles, roughly at right angles to GLORIA's track, have established that these echoes come from steep slopes on the sea floor (ref. 2, and R. B. Whitmarsh and A. S. Laughton, unpublished). Many of these echoes have a gentle curvature to the right as they approach fracture zone valleys, as illustrated in the north-eastern quadrant of Fig. 2. Other echoes have a more NE–SW trend and are oriented at less than 50° to the fracture zone valley. These echoes cut across the 020° lineations, giving the impression of an abrupt change in direction which often approximately coincides with the fracture zone escarpment, as can be seen in the south-eastern quadrant of Fig. 2.

The first order relief in the crestal zone of a slow-spreading ridge is tectonically controlled[3-5]. Volcanic processes give rise to only relatively short ($\lesssim 5$ km)[6] and relatively low linear ridges and occasional isolated shield mountains. The above evidence, plus the fact that many echoes are attributable to local steepening of slopes rather than to ridges, suggests that most linear echoes on the mosaic are attributable to fault scarps. Furthermore, near-bottom observations within the median valley have demonstrated that scarps of large normal faults are exposed there[7]. Thus we may use the echoes on the mosaic as a basis for discussing the tectonics of a slow spreading ridge crest. At the same time an explanation is sought for (1) the gently curved fault outcrops and (2) the set of faults which trend obliquely to them.

Fig. 1 Bathymetry of the Mid-Atlantic Ridge near 37 °N (FAMOUS area), contour interval 100 fathoms, depths < 1,000 fathoms are stippled (courtesy J. D. Phillips[16]). Box is area of Fig. 2.



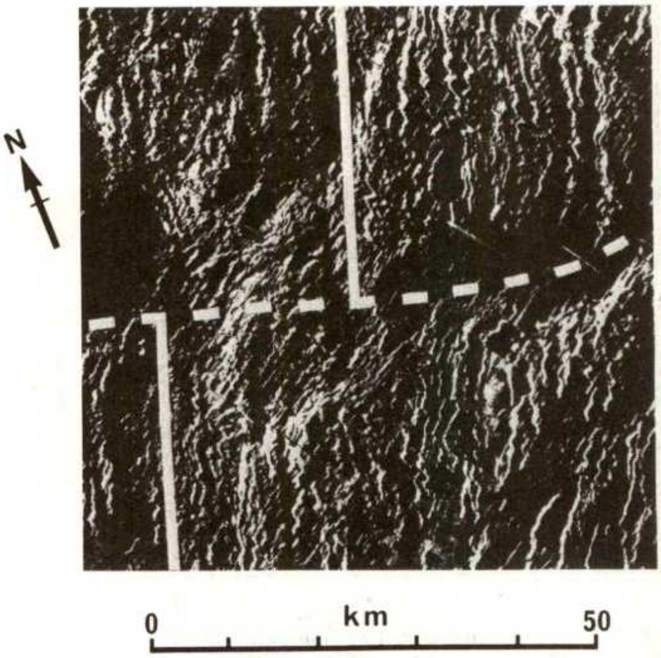


Fig. 2 Part of the GLORIA sonograph mosaic from the box in Fig. 1. The median valley and fracture zone B axes are indicated by continuous and dashed lines respectively. The sinusoidal nature of some targets is an artefact from the yaw of the sonar vehicle.

Figure 3*a* is a schematic representation of the fault trends (echoes) in the area of Fig. 2. In the mosaic the fault scarps lying east of the median valley are better delineated because they face predominantly inwards, and thus towards GLORIA; those lying west of the axis are rarely insonified. For this discussion, however, it is assumed that there is rotational symmetry between east and west, as shown in Fig. 3*a*.

The gently curved and oblique faults cannot be explained by drag across the transform fault section of the fracture zone because the sense of drag is wrong. Nor can the gentle curvature in the NE and SW quadrants of Fig. 3*a* be attributed to the intersection of fault planes, striking parallel to the median valley and dipping towards its centre, with the cross-cutting valley of the fracture zone, since, even if faults are proposed which have hades much less than are actually observed[7], it is not possible to obtain a fault trace of approximately the correct curvature, given the bathymetry of fracture zone B, nor is this model consistent with the morphology in the other two quadrants (Fig. 3*b*).

If the fault surface is no longer constrained to be a plane, but the strike lines are still straight and parallel to the median valley, a non-planar subterranean fault surface can be derived from the fault trace on the sea bed and the sea-bed contours. The resulting surface is non-cylindrical and has a near vertical dip on high ground but reduces rapidly to about 10° at the level of the floor of the transform fault valley. Even if a cylindrical surface were forced through our observations the horizontal motion towards the median valley at the foot of a fault block would create difficult geometrical problems in a quasisteady-state sea-floor spreading model.

We prefer to explain the gently curved fault traces as resulting from steeply dipping faults resulting from caldera subsidence at the centre of the median valley. This hypo-

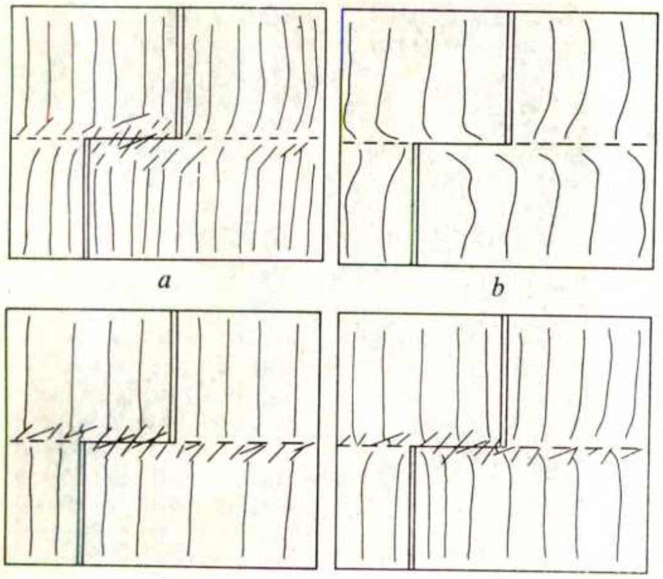


Fig. 3 *a*, Schematic sketch of fault trends observed in Fig. 2. *b*, Fault pattern due to a set of inward dipping low angle faults striking parallel to the median valley. *c*, Fault pattern which would be associated with wrench faulting along the transform fault together with vertical normal faults striking parallel to the median valley. *d*, Fault pattern produced by a combination of the processes of caldera subsidence and wrench faulting (see text).

thesis differs fundamentally from the previous ones in that the faults are not assumed to have had an initial strike parallel to the median valley axis along their whole length. The curvature reflects the shape of the underlying magma chamber at the time of collapse.

An analogy is drawn here with the elliptical caldera faults common within central vent volcanoes[8] and with spindle-shaped graben (*fuseau alongé*) described in Afar[9]. Intermittent caldera subsidence has recently been invoked to explain features of the natural seismicity in the median valley[10]. The shape of the caldera faults can be explained if there is a cigar-shaped magma chamber beneath and parallel to each median valley segment. When the magma chamber pressure falls, the overlying lavas subside along near vertical fault planes, curved in plan, which outline the caldera. The axial intrusion zone, however, in which volcanic extrusives accumulate at the seafloor, remains a strip of uniform width between the caldera faults so that, for example, magnetic reversal boundaries remain straight.

The caldera subsidence model requires that, near fracture zones, faults should curve towards the median valley in all four quadrants in Fig. 3*a*. This is not seen on the mosaic in the NW and SE quadrants possibly because the trends would be nearly normal to GLORIA's track. It can be detected, however, in part of the NW and SE quadrants on a very detailed bathymetric chart of the median valley floor (ref. 16 and J. D. Phillips, unpublished charts) in the form of curved ridges and scarps.

Within the offset region between the valley axes, the caldera faults in the NW and SE quadrants seem to be disrupted and offset by the second set of faults which are oriented at < 50° to the fracture zone. The latter faults represent the normal and shear faults associated with wrench faulting along the transform fault. These are well known both from clay model experiments[11–13] and from field work on land[13]. Similar faults exist along a transform fault zone in central Afar[14]. Along wrench fault zones, normal faults are developed at 30–50° to the main fault, and shear faults (Riedel shears) at angles < 20°. Trends of both relative orientations can be distinguished in the sonar mosaic and were also found by Detrick *et al.*[15] in a detailed sonar study near the 37°N fracture zone (Fig. 1). In a situation of steady-state seafloor spreading these faults would give rise to the pattern in Fig. 3*c*. Each oblique fracture generated along the transform fault is eventually cut in half by this fault and the halves are carried away in opposite directions by seafloor spreading.

When these two tectonic processes are combined in a steady-state seafloor spreading model, the fault pattern of Fig. 3*d* is obtained. As the curved caldera subsidence faults in the NW and SE quadrants are carried away from the valley axis they become increasingly dissected and offset by the shear faults associated with wrench faulting, and thus are not detected there as continuous features.

A consequence of this model is that the pressure of magma in the chamber supplying the injection zone should pulsate in such a way that new caldera faults are produced at least as often as every 160,000 yr, according to the spacing between caldera faults. Uplift of the ridge crest over a width of several kilometres occurs as pressure increases and this is followed by more localised collapse along caldera faults, when pressure falls, giving rise to the median valley topography.

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Post-oxide phases of olivine and pyroxene and mineralogy of the mantle

I REPORT here experimental confirmation of the prediction[1] that oxide mixtures with either olivine or pyroxene stoichiometries are not stable mineral assemblages under lower mantle conditions. Pyroxene with a few percentages of Al_2O_3 transforms into the garnet structure at high pressure[2]. Further I have found that garnets rich in pyrope transform to the orthorhombic perovskite phase at loading pressures above 270 kbar and temperatures above 800 °C. For those reasons, and in order to allow the sample to absorb the laser beam with maximum efficiency, I used a glass form of 95% by weight $(Mg_{0.75}Fe_{0.25})SiO_3 + 5\%$ (weight) Al_2O_3 as a starting sample. This material is equivalent to a mixture with 93.7 mol % of pyroxene plus 6.3 mol % of pyrope-rich garnets, and is identical to that studied by Ringwood and Major in their synthesis of the garnet phase[3].

Fine powder of the starting material was compressed in a diamond-anvil press fitted with a lever and spring assembly and was irradiated by a cw YAG laser while the sample was maintained under steady pressure. The quenched sample was removed to a modified 57.3 mm Debye–Scherrer camera for X-ray diffraction study. The details of the experimental procedure have been described elsewhere[4].

X-ray diffraction data for the glass sample quenched from a loading pressure of about 260 kbar and temperature of about 1,500 °C are shown in Table 1. The pattern can readily be indexed as a mixture of garnet (with a lattice parameter

$a_0 = 11.502 \pm 0.013$ Å), spinel (with $a_0 = 8.113 \pm 0.017$ Å), stishovite, and the orthorhombic perovskite phase (with $a_0 = 4.799 \pm 0.008$, $b_0 = 4.959 \pm 0.008$, $c_0 = 6.905 \pm 0.011$ Å, $Z = 4$, and possible space group of Pbnm). The lattice parameter for the garnet is identical to that reported by Ringwood and Major[3]. I suggest that the garnet (which is not the same garnet as those of pyrope–almandite solid solutions found in nature) has disproportionated into a mixture of spinel plus stishovite; a slight amount of Al_2O_3 may be retained in the garnet or may exist as a separate phase of corundum which may not be detectable in the X-ray pattern. Spinel and stishovite (perhaps Al_2O_3 too) may further combine to form the perovskite phase.

Among the 36 observed reflections in Table 1, 30 can be indexed on the basis of an orthorhombic perovskite structure. Out of the 30, 16 are not interfered with by reflections of other observed phases. The relative intensities for strong reflections of the observed and calculated orthorhombic perovskite phase of $ScAlO_3$ (ref. 5) and $MnVO_3$ (ref. 6) are also listed in Table 1 for comparison. The general agreement is remarkable.

Could the hypothesised metastability be attributable to the fact that the perovskite phase was synthesised from a glass? In dismissing that possibility, I used an intimate mixture of olivine $(Mg_{0.75}Fe_{0.25})_2SiO_4$ with amorphous SiO_2 and Al_2O_3 in the same proportion as in the glass sample. With a few weak reflections corresponding to olivine, similar results (Table 1) were obtained in the mixture sample under the same P,T conditions. With increasing pressure, the phase transformations observed in the $(Mg_{0.75}Fe_{0.25})SiO_3 + Al_2O_3$ system are inferred to be: garnet→spinel + stishovite + Al_2O_3(?)→ perovskite.

To examine the pyroxene system two samples with the pyroxene stoichiometry were studied at 280 kbar and 1,500 °C. One is the intimate mixture of $(Mg_{0.75}Fe_{0.25})O$ with silicic acid in the pyroxene proportion; the other is a glass form of $(Mg_{0.7}Fe_{0.3})SiO_3$. The latter is identical to that used by Ringwood and Major[2].

The perovskite phase has been observed in both samples. The X-ray diffraction data for the mixture sample comprise three phases (ferropericlase, stishovite, and perovskite). The lattice parameters are $a_0 = 4.242 \pm 0.001$ Å for ferropericlase and $a_0 = 4.793 \pm 0.004$, $b_0 = 4.937 \pm 0.004$, and $c_0 = 6.889 \pm 0.006$ Å for perovskite. The molar volume for the perovskite phase of $(Mg_{0.75}Fe_{0.25})SiO_3$ thus calculated is 24.58 ± 0.04 cm^3 mol^{-1}, which is 3.7% smaller than that of its isochemical mixture $(Mg_{0.75}Fe_{0.25})O + SiO_2$ (stishovite). The latter value, 3.7%, is identical to that observed in the perovskite phase of $MgSiO_3$ (ref. 7).

The results obtained in the glass sample of $(Mg_{0.7}Fe_{0.3})SiO_3$ are almost identical to those for the olivine system except for the absence of the olivine phase and for strong reflections from stishovite.

Table 1 X-ray diffraction data (room temperature and 1 bar pressure) for a glass of 95% (weight) $(Mg_{0.75}Fe_{0.25})SiO_3 + 5\%$ (weight) Al_2O_3 quenched from a loading pressure of about 260 kbar and temperature of about 1,500 °C (CoKα)*

Observed		Garnet			Spinel $(Mg_{0.75}Fe_{0.25})_2SiO_4$			Stishovite SiO_2			Perovskite		
I/I_{100}†	d(Å)	d(Å)	hkl	I/I_{100}	d(Å)	hkl	I/I_{100}	d(Å)	hkl	I/I_{100}	d(Å)	hkl	I/I_{100}
30	3.45										3.45	110,002	
35	3.09										3.09	111	20
50	2.96							2.96	110	100			
50	2.88	2.88	400	60	2.87	220	25						
80	2.576	2.572	420	100									
20	2.477										2.480	020	18
100	2.443				2.446	311	100				2.440	112	100
20	2.400										2.399	200	20
30	2.344	2.348	422	30	2.342	222	5				2.334	021	
40	2.259	2.256	510	30				2.25	101	18	2.266	201	
10	2.198										2.203	120	
30	2.155										2.160	210	
50	2.090	2.100	521	20				2.09	200	1	2.088	013	
30	2.062										2.061	211	30
30	2.011				2.028	400	50				2.014	022	
30	1.978							1.98	111	35	1.970	202	
40	1.920	1.917	600								1.915	113	
45	1.863	1.866	611	40				1.87	210	13	1.857	122	
40	1.737	1.734	622										
70	1.723										1.726 1:724	004 220	55
5	1.690										1.687	023	
5	1.679										1.673	221	
5	1.661	1.660	444	30							1.661	203	
<5	1.635										1.630	014	
40	1.596	1.595	640	60							1.600 1.592	300 123	
20	1.559				1.561	333	25				1.558	301	
50	1.537	1.537	642	100				1.53	211	50			
20	1.523										1.524 1.522	131 310	20
<5	1.483							1.478	220	18	1.491 1.487	032 311	
10	1.438	1.438	800	30	1.434	440	65						
30	1.421										1.424	132	
10	1.405										1.401	204	20
30	1.395										1.393	312	35
<5	1.254	1.255	842	60							1.253	322	
<5	1.237				1.237	533	10	1.235	301	25	1.240	040	
15	1.219							1.215	112	9	1.220	041	

*Data for garnet, spinel, stishovite and perovskite were obtained from the following lattice parameters and other relevant literature: Garnet—$a_0 = 11.502$ Å, the relative intensities (I/I_{100}) are assumed to be similar to those of pyrope-rich garnet. Spinel—$a_0 = 8.113$ Å, I/I_{100} were estimated from ref. 11. Stishovite—from ref. 12. Perovskite—$a_0 = 4.799$, $b_0 = 4.959$, and $c_0 = 6.905$ Å; I/I_{100} are those of strong reflections of orthorhombic perovskite phase of $ScAlO_3$ (ref. 5) and $MnVO_3$ (ref. 6) for comparison.

†Estimated visually.

Table 2 Mineralogical zones for the mantle and their major mineral phases based on the quenched static high pressure and temperature experimental results. See text for reference for each phase transition

	Depth (km)	Major	Mineral	Phases
	33			
Upper mantle (zone B)	Olivine zone	Olivine	Pyroxene $+Al_2O_3$	Garnet
	350		Garnet	
Transition zone (zone C)	Spinel zone	β-phase Spinel	$-Al_2O_3$ Spinel + Stishovite Ilmenite	Ferropericlase + unknown (I)
	650			
	Oxide zone	Ferropericlase + stishovite	Ferropericlase + stishovite	Ferropericlase + unknown (II)
	1,000			
Lower mantle (zone D)	Perovskite zone	Perovskite + ferropericlase	Perovskite	Perovskite
	2,800			

Phase transformations in this system, with increasing pressure, are inferred to be pyroxene→spinel+stishovite→ilmenite→ferropericlase+stishovite→perovskite.

For the olivine system two samples of single phases of $(Mg_{0.8}Fe_{0.2})_2SiO_4$ and $(Mg_{0.7}Fe_{0.3})_2SiO_4$, which are identical to those used by Ringwood and Major[8], were studied. The quality of the X-ray diffraction data for the olivine sample of $(Mg_{0.7}Fe_{0.3})_2SiO_4$ are extremely good. A total of 52 reflections, which correspond to five crystal phases, has been observed. The lattice parameters are $a_0 = 4.774 \pm 0.008$, $b_0 = 10.354 \pm 0.016$, and $c_0 = 6.041 \pm 0.010$ Å for the starting sample of olivine; $a_0 = 8.143 \pm 0.007$ Å for the spinel phase; $a_0 = 4.278 \pm 0.003$ Å for ferropericlase; and $a_0 = 4.805 \pm 0.004$, $b_0 = 4.949 \pm 0.004$, and $c_0 = 6.922 \pm 0.006$ Å for the perovskite phase of $(Mg_{0.7}Fe_{0.3})SiO_3$. These yield a molar volume of the perovskite phase of 24.78 ± 0.06 cm^3 mol^{-1} which is 4% smaller than that of its isochemical mixture of oxides.

The most abundant phases found in Fe_2SiO_4 (ref. 9) and Mg_2SiO_4 (ref. 10) are the rocksalt phase and stishovite. I found that ferropericlase (rocksalt) and perovskite are the most abundant phases and only a trace amount of stishovite can be identified on the basis of the relative intensities of the various phases. I therefore conclude that nearly all the stishovite has reacted with ferropericlase to form perovskite in the present experiment. On the basis of all the available evidence, the following phase transformations are inferred to have occurred in the olivine system: olivine→β phase→spinel→ferropericlase+stishovite→ferropericlase+perovskite with increasing pressure.

Similar results were obtained in the olivine sample of $(Mg_{0.8}Fe_{0.2})_2SiO_4$. Since this sample contained less iron than the previous one, it did not absorb the laser beam very well and was not well heated. Only about 30% of the sample has been transformed to the perovskite phase to judge from the relative intensities of the X-ray diffraction pattern. In a later run, however, the sample was intimately mixed with a few per cent of powdered graphite, which served to absorb the laser irradiation and to heat the sample under compression. The results show that sample to be about 80% transformed. The lattice parameters for the perovskite phase of $(Mg_{0.8}Fe_{0.2})SiO_3$ are $a_0 = 4.796 \pm 0.004$, $b_0 = 4.943 \pm 0.004$, and $c_0 = 6.902 \pm 0.006$ Å.

I would suggest that the mantle be subdivided on the basis of its major mineral phases or chemicals with depth, rather than on the basis of velocity distributions (Table 2). Of course, the boundaries between the successive phases shown in Table 2 should in each case comprise a mixture of the phase above and that below the boundary for the ferromagnesian solid solution system. In view of all the experimental evidence, I conclude that the lower mantle is composed mainly of the perovskite phase, which may represent the last stage of the mineralogical evolution in the Earth's mantle.

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Ignition by shock wave focusing and staging of thermonuclear microexplosions

FROM the beginning of laser fusions research[1] the main problem has been the very large size of the ignition apparatus. For this reason relativistic electron beams[2] and, more recently, ion beams[3] have been proposed as alternatives. Relativistic electron beams have the following principal problems, however, when compared with laser beams: first the pulse length is rather long, and second the beam focusing is not as good. Ion beams are not as easily focused as either laser or electron beams. Furthermore, in both laser- and electron-beam induced implosion most of the energy goes into the ablation products, rather than the thermonuclear target. I propose here an alternative, non-ablative energy deposition method, applicable to laser,

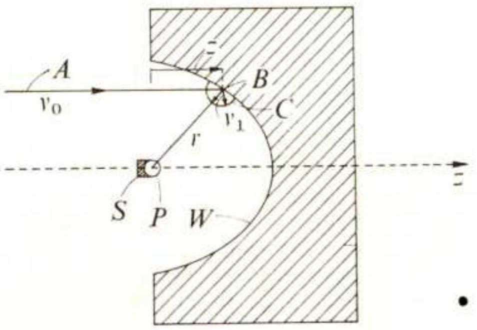


Fig. 1 A beam ray A hits a concave wall W, at point B. The wall material is ablated forming a small spherical shock wave C. The superposition of all the shock waves from all beam rays results in a curved shock wave, by Huygens' Principle. The beam consists of all the rays striking the curved wall and its total cross section is equal to the wall diameter.

electron or ion beams, and which has the following distinct advantages: (1) The energy is delivered to the target by a shock wave. (2) The beam energy can be focused precisely on the target. (3) The energy pulse length can be shortened by about an order of magnitude. (4) The method permits staging, whereby a small microexplosion can set off a larger one.

The principle of the idea is explained in Fig. 1. If the wall is properly shaped the resultant shock wave will converge on to the thermonuclear pellet (P). The pellet is protected by the shield (S) against the in coming beam, to prevent it from being preheated, which would hinder its compression to high densities. The shield simultaneously serves as an anvil for the pellet.

The wall shape is approximately determined by Fermat's Principle, and in a r–z coordinate system, with $r = z = 0$ at the pellet centre the wall shape would be given by

$$t = (z/v_0)+(r/v_1) = \text{const.} \tag{1}$$

where v_0 is the velocity of the incoming beam particles and v_1 the shock velocity. For relativistic electron beams $v_1 \ll v_0$, and hence the wall shape should be hemispherical. For ion beams, one may have $v_0 \simeq v_1$, and the wall should be paraboloid.

By a proper layered variation of the density ρ and the atomic weight A in the wall material a shock wave is obtained which becomes radially compressed as the shock approaches the centre. If a is the initial distance from the centre of convergence of a particle immediately after the shock has been formed and if all the fluid particles arrive simultaneously at time $t = t_0$ at the centre of convergence (which is the condition for the radial shock wave compression), then the initial velocity of the particle in the shock wave must be

$$v(a) = a/t_0 \tag{2}$$

The particle trajectory is then given by

$$r = a(1-(t/t_0)) \tag{3}$$

and the fluid density in the shock wave changes according to

$$\begin{aligned}\rho &= \rho_0(a)(a/r)^3\\ &= \rho_0(a)(1-(t/t_0))^{-3}\end{aligned} \tag{4}$$

The energy flux density at fixed r, $r = r_1$, is then given by

$$\begin{aligned}\varepsilon_1 &= (1/2)\rho v^3\\ &= (1/2)\rho_0(a)(r_1/t_0)^3(1-(t/t_0))^{-6}\end{aligned} \tag{5}$$

To achieve adiabatic high density compression the required[4] pulse shape is

$$\varepsilon \propto (1-(t/t_0))^{-15/8} \tag{6}$$

This can be obtained by making

$$\begin{aligned}\rho_0(a) &\propto (1-(t/t_0))^{33/8}\\ &= (r_1/a)^{33/8} \propto a^{-33/8} \simeq a^{-4}\end{aligned} \tag{7}$$

If the temperature T in the shock wave is uniform, then the initial fluid velocity is

$$v^2(a) \propto T/A \tag{8}$$

where A is the atomic weight of the ablated material. But since $v(a) \propto a$ it follows that

$$A \propto a^{-2} \tag{9}$$

Assume an initial shock thickness of $\Delta a = 2$ cm, and an initial distance of the shock front from the centre $r_1 = 1$ cm; this would then require a variation in ρ of $\Delta\rho/\rho = (\Delta a/r_1)^4 = 16$, and a variation in the atomic weight by $\Delta A/A = (\Delta a/r_1)^2 = 4$. These variations in density and atomic weight can easily be met by putting several layers of different materials on the surface of the curved wall.

As the shock wave approaches the centre the energy flux profile remains self-similar and the energy flux density increases as $\varepsilon \propto r^{-3}$, with geometric convergence contributing a factor r^{-2}, and radial wave bunching the additional r^{-1}. If the shock is focused from $r_1 = 1$ cm down to $r_0 = 0.1$ cm, the energy flux density increases by a factor 10^3, and the pulse length is reduced by a factor 10. If an electron beam is used with a typical pulse length of 5×10^{-8} s, the reduced pulse length at the target would be only 5×10^{-9} s.

If the blast wave from one microexplosion is now focused by another solid wall on to a second thermonuclear target, a larger microexplosion can be ignited. In turn, the blast wave of the second microexplosion by reflection from a third wall can ignite a third, even larger microexplosion. The energy gain E_1/E_0 for one microexplosion is approximately given by (E_0, E_1 in J)

$$E_1/E_0 \simeq E_0^{1/3} \tag{10}$$

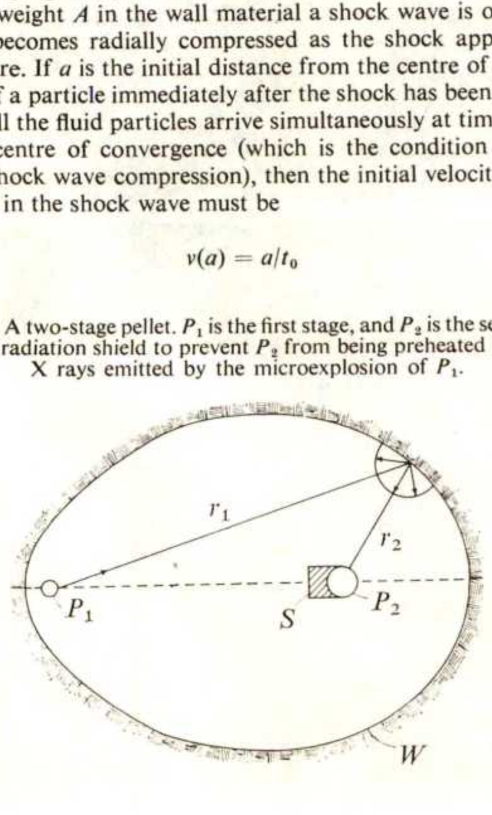
where E_0 is the input energy. For a TD thermonuclear reaction, only the energy going into charged fusion products can be utilised (which is about 20%). Assuming furthermore a coupling efficiency for this process of ~50% gives

$$E_1/E_0 \simeq cE_0^{1/3}, \quad c \simeq 0.1 \tag{11}$$

The energy gain in an n-stage microexplosion (allowing a factor 5 for the energy released by neutrons) is

$$E_n = 5\sum_{n=0}^{n} c^{\sum_{m=0}^{n-1}(4/3)^m} E_0^{(4/3)^n} \tag{12}$$

Fig. 2 A two-stage pellet. P_1 is the first stage, and P_2 is the second. S is a radiation shield to prevent P_2 from being preheated by the X rays emitted by the microexplosion of P_1.



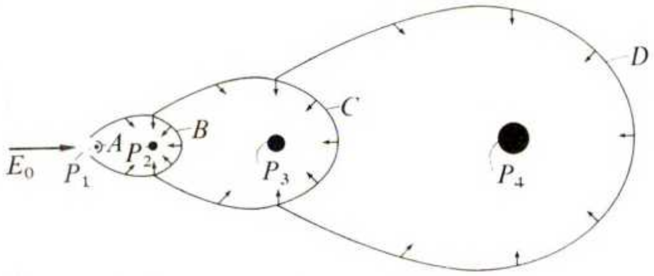


Fig. 3 A four-stage pellet. The incoming beam carries energy E_0, and ignition P_1. The wall A, cannot withstand the explosion of P_1, which then is reflected by the wall B, to ignite P_2, and so on.

If for example $E_0 = 10^4$ J, then a four-stage pellet would have $E_4 = 8\times10^6$ J. For $E_0 = 10^5$ J a two-stage pellet would already give $E_2 = 2\times10^7$ J. These values are at the threshold of reactor operation and show the great importance of staging in reducing the initial energy input.

Figure 2 shows a two-stage pellet, with both pellets located inside an egg-shaped cavity. The equation of the cavity wall is obtained from Fermat's Principle, and thus for the wall surface in bipolar coordinates r_1, r_2 centred at P_1 and P_2

$$(r_1/v_1)+(r_2/v_2) = \text{const.} \tag{13}$$

where v_1 is the velocity of the blast wave from pellet P_1, and v_2 the velocity of the reflected shock. Figure 2 is drawn for the example $v_2 = (1/2)v_1$. To ignite P_1 any of the proposed methods can be used, including that proposed here. For this, however, the cavity must have openings near P_1, to allow the passage of the original beams to ignite P_1. A 4-stage pellet is shown in Fig. 3. The walls A, B and C are strong enough to withstand the initial blast wave but weak enough to collapse at the subsequent strong ones.

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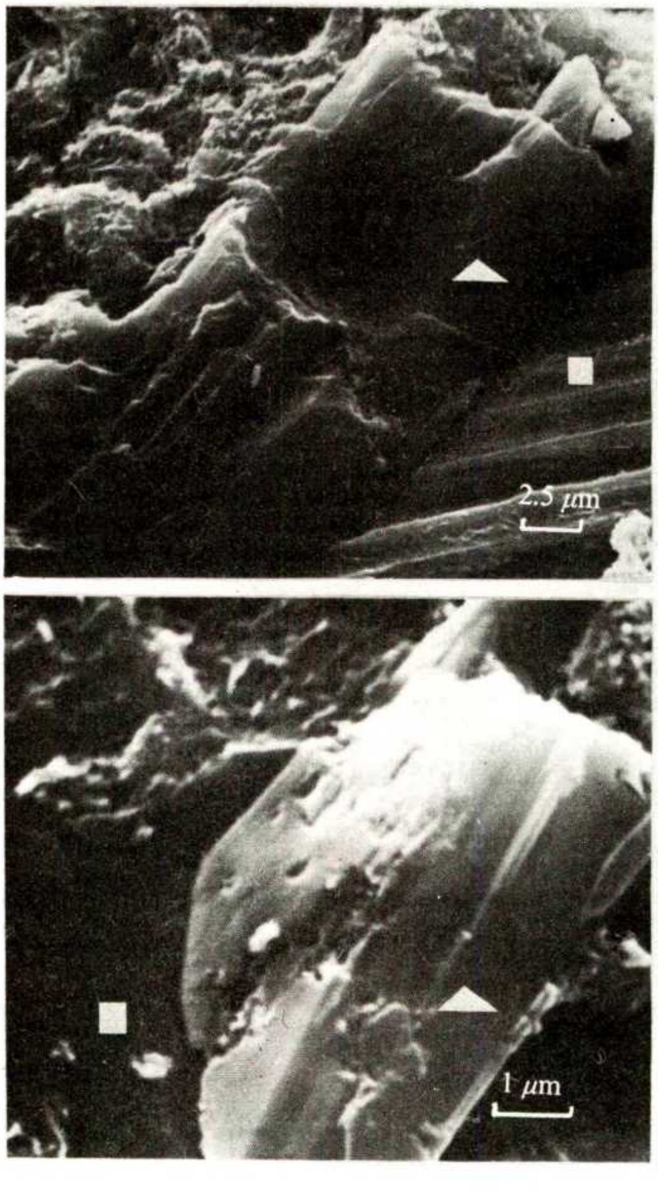
Mechanism of corrosion protection in reinforced concrete marine structures

RECENT developments in concrete offshore structures have drawn attention to a disturbing ignorance of the mechanism by which steel corrosion is prevented when it is embedded in dense concrete[1]. The traditional view has been that the metal remains passivated owing to the high pH (~ 12.5) of the pore solution in concrete. It is, however, well known that relatively small concentrations of Cl^- destroy the corrosion inhibitive properties of non-buffered alkalis and ~ 700 p.p.m. of Cl^- will depassivate steel in limewater at pH 12.5 (ref. 2). Since seawater contains concentrations of Cl^- far in excess of this figure, and since penetration by these ions to the reinforcing bars in concrete takes place within a small fraction of the service life of a marine concrete structure[3], corrosion might be expected to be a frequent problem. In practice, however, there are many examples of reinforced concrete structures which have remained durable in seawater for 30 yr and more. Such corrosion problems as have been reported are, in the majority of cases, associated only with areas immediately above the level of high tide, where the concrete is not maintained in a saturated condition.

These observations have led to an alternative suggestion that the main reason why saturated concrete suppresses the corrosion of steel is not its ability to promote anodic passivation but its low permeability to dissolved oxygen. Thus, although the ingress of Cl^- from seawater destroys the passive film on steel, a high degree of polarisation of the cathodic reaction prevents an appreciable corrosion current from being sustained[4]. Although this view surmounts the problem of Cl^- tolerance, it is by no means free from objections. It fails to provide an adequate explanation for the small number of cases in which corrosion of reinforcement has arisen in fully submerged marine concrete[3]. Furthermore, it has been observed that steel which is free from corrosion in concrete saturated with seawater is characterised by a high electrode potential, more positive than the passivation potential, and that the surface of the metal remains bright and apparently passivated[5].

I suggest that these phenomena may be more satisfactorily explained if account is taken of the nature of the interfacial zone separating concrete and embedded steel reinforcement. Evidence has been obtained that this region of the material is composed largely of segregated

Fig. 1 Scanning electron micrographs of fracture surfaces showing interfacial lime layer (△) in contact with steel (□).



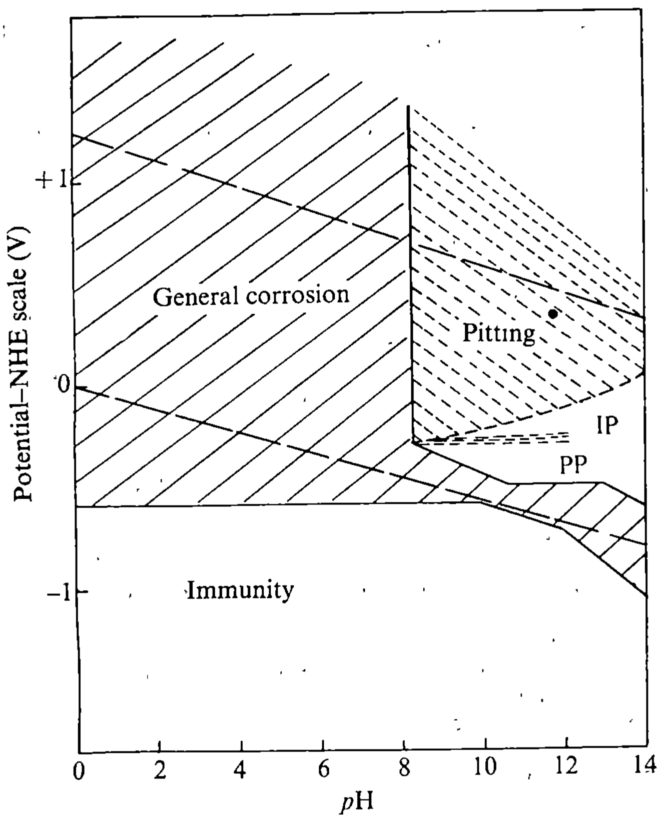


Fig. 2 Potential/pH diagram for Fe in 1 M Cl^- solutions. PP, Perfect passivity; IP, imperfect passivity

lime[6,7]. Recent studies of the interface with a scanning electron microscope have confirmed this and shown the metal to be in intimate contact with a lime-rich layer over most of its surface (Fig. 1.) This layer will modify the electrode characteristics of the metal: The effect on the cathodic reduction of oxygen is apparent, since a considerable fraction of the metal surface is screened from the direct access of oxygen. The influence on the anodic behaviour of the exposed regions of the steel is also likely to be significant since the surrounding lime provides a reservoir of OH^- in direct proximity to the metal. Thus, the tendency for hydrolysis of anodic reaction products to depress the local pH will promote dissolution of the adjacent lime, thereby exerting a form of local buffering around the anodic sites.

It is established that anodic passivation of iron is greatly facilitated in the presence of alkaline buffers For example, borate solutions at pH 9 are efficient corrosion inhibitors because their buffering action stabilises the anodic formation of $Fe(OH)_2$ and γ-Fe_2O_3 which reinforces the passivating oxide film on iron at regions of localised weakness[8]. Solutions in which OH^- is the sole anion do not exhibit this behaviour at pH values lower than about 12 because of their lack of buffering capacity. In the presence of Cl^-, the effect of alkaline buffering may be understood in terms of the modified Pourbaix diagram for iron (Fig. 2)[9]. This shows that the domain of passivity covers a band of potentials, the width of which diminishes rapidly with decreasing pH. Thus, in concrete saturated with seawater, the buffering effect of the lime layer may preserve the conditions at a local anode within the confines of this domain and, in view of polarisation of oxygen reduction, it is likely that the potential will be maintained in the range of 'perfect passivity' where pits in the oxide film are unstable. Corrosion is normally observed only in conditions where the material is subjected to alternate wetting and drying when evaporation can produce very high local salt concentrations and intimate contact between the lime layer and the metal may break down.

If the postulated role of the interfacial zone is accepted, several other practical observations become readily explicable. Measures which promote homogeneity of the interface, such as the application of a slurry of neat cement paste to the surface of reinforcing bars before surrounding them with concrete, are effective in reducing the risk of corrosion[10]. Conversely, it has been found that voids at the interface are almost invariably the positions at which corrosion of steel reinforcement and prestressing tendons takes place in the presence of high Cl^- concentrations[11]. Furthermore, since the lime component of hydrated Portland cement is vulnerable to carbonation, leaching and attack by ions such as SO_4^{2-} and Mg^{2+}, the integrity of the interfacial zone depends on its being shielded from these forms of degradation by surrounding the reinforcement with an adequately deep covering of good quality concrete. This is the time-honoured method of providing corrosion protection to reinforcing steel in concrete. It is noteworthy that reported instances of reinforcement corrosion in concrete, fully submerged for extended periods in seawater, have been accompanied by observations of substantial chemical attack of the hydrated cement paste[12].

Further experiments are in hand to establish the effect of the interfacial layer on the electrode characteristics of steel in concrete, and an account of factors affecting the microstructure of the interfacial zone will be published elsewhere (M. Al Khalaf and C. L. Page, in preparation). It is important that the fundamental basis for the corrosion behaviour of this system should be more fully understood so that rational assessments may be made of the potential risks of damage to concrete offshore structures. In particular, it is conceivable that the effects of cumulative fatigue loading due to wave action on the highly stressed elements, which are a novel feature of current designs, may stimulate gradual chemical degradation and leaching of the interfacial lime layer. The possible consequences of this on the fatigue life of the reinforcing bars are not readily predictable from accelerated corrosion fatigue tests of the types presently being conducted[5].

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Conodonts as indicators of palaeogeographic regimes

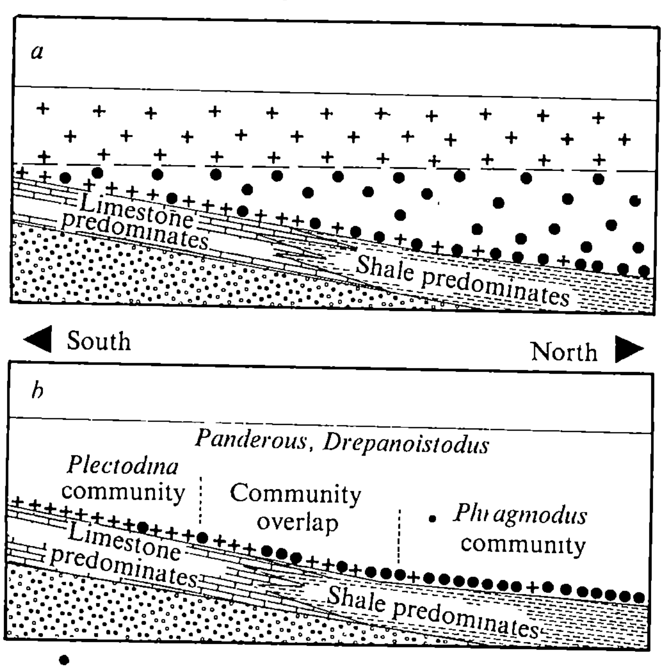
CONODONTS are disjunct elements of, probably, an internal food-gathering and/or macerating device of the conodontophorid animal which flourished in the Palaeozoic and early Mesozoic seas. They are excellent tools for biozonations and long distance correlations of marine sedimentary rocks of that age[1]

Here, we draw attention to the potential of conodonts as tools for palaeogeographic analyses of marine depositional environments. Ordovician conodonts can be used in studies involving the extent and relative depths of sedimentary basins; for unravelling patterns of transgressions and regressions; as aids in the recognition of depositional environments characterised by raised temperature and salinity; and in understanding major palaeogeographic and tectonic changes. These new aspects of conodont studies are based on the hypothesis that the majority of conodontophorids were nektobenthic organisms[2] rather than pelagic[3].

Two major ecological models for the conodontophorids have been proposed (Fig. 1*a* and *b*). The first[3] (Fig. 1*a*) suggests that the conodontophorids were pelagic free-swimming organisms with certain taxa exhibiting depth stratification in the water column Thus, nearshore deposits would yield only surface-dwelling forms, and progressively deeper water facies would yield surface-dwelling forms plus elements from deeper 'stacked' biofacies. The second model (Fig. 1b), which we favour, suggests that the conodontophorids were primarily nektobenthic and laterally segregated into series of well defined communities; this model is applicable to all major types of Ordovician marine depositional environments[2], to epeiric sea deposits, and to marginal cratonic basin and continental margin deposits. Studies of Carboniferous[5], Silurian, and Lower Devonian conodonts have indicated similar patterns of distribution and we believe our model to be generally applicable to the majority of conodontophorids existing from the Ordovician through to the Triassic Cambrian forms we are still uncertain about.

Samples from epeiric-sea deposits frequently indicate extensive overlap between neighbouring conodontophorid communities (Fig. 2) We believe that occurs because of the exceedingly gradual depth changes of the epeiric sea. We also believe, however, that the inherent time-average nature of the conodont sample is a contributory factor. Whether a conodont collection will represent more than one community is of course directly dependent on rates of sedimentation, stratigraphic thickness of the sample, and, ultimately, the stability of the depositional environment. This is of particular importance in studies of conodonts digested from starved sequences such as the Lower and Middle Ordovician of Sweden where net average sedimentation rates of less than 5 mm kyr^{-1} have been estimated[6,7].

Fig. 1 Contrasting models of conodontophorid habit: *a*, pelagic; *b*, nektobenthic. Arrow, sea level; +, *Plectodina*, *Icriodella* and *Panderodus*; ●, *Phragmodus*, *Amorphognathus* and *Belodina*. We prefer model *b*.



We believe the overlap of neighbouring conodontophorid communities can be used to identify minor transgressions and regressions in a sequence, of strata simply by comparing the proportions of those living in shallow water with those in deeper water (see Fig. 2). We have compared the proportions of conodont elements from offshore living *Phragmodus* with those of nearshore living *Plectodina* from a series of samples from two sections of the Champlainian and early Cincinnatian Lexington Limestone of Kentucky. The data are based on numbers of conodont elements tabulated by Bergström and Sweet[8].

The *Phragmodus* apparatus includes four different elements (Fig. 2*a*I) whereas the *Plectodina* apparatus includes six different elements (Fig. 2*a*II), so the numbers could be considered biased in favour of the *Plectodina* community. Though the exact numbers of elements in each of these two apparatuses are not known, only the proportional distributions and trends through time are of interest (Fig. 2*b* and *c*). A moving average minimises the element of chance (in this case samples with possible artificially biased conodont collections), and emphasises the trend.

If we are correct in our assumption about the nektobenthic habit of *Phragmodus* and *Plectodina*, then the resulting curves of proportions of *Phragmodus* elements should directly reflect transgressions with increasing percentage and regressions with decreasing percentage. If such a relationship existed it should show up as a clearly displayed trend; (this, of course, with the proviso that sampling intervals are not too large and sedimentation rates are not too slow). Such trends are clearly visible in Fig. 2 (for example, the initial transgression in section 64S3 (Fig 2*c*), which is based on six samples showing almost a linear relationship, or the final major transgression interspersed with a series of minor regressions in the Clays Ferry section (Fig. 2*b*)). If the conodontophorids were pelagic the plotted values would be unevenly distributed with a number of unrelated maxima and minima.

The Lexington Limestone is a bioclastic calcarenite with a varying amount of argillaceous admixture and shaly interbeds, and exhibits frequent facies changes and recurrence of facies types[9]. In other words, it is characterised by numerous fluctuations in water level[10]. Bergström and Sweet[11] noted that *Phragmodus* is more common in the shaly intervals, and Seddon and Sweet[3] have suggested that the ratio between *Phragmodus* and *Plectodina* provides a crude index to relative water depth. We believe that ratio can be used with much more reliance and precision; that is, to determine possible changes in water level, bed by bed in a section.

A more stringent test of our model is provided by an examination of a deeper basin of considerable size to determine what the supposed surface-dwelling forms also accumulate with those deeper in the water column. Chamberlain and Clark[12] have studied conodonts and trace fossils from the Oquirrh Basin of central Utah where up to 8,000 m of sediments accumulated during the Pennsylvanian and early Permian on the eastern margin of the Cordilleran geosyncline The sediments and the trace fossil sequence—*Cruziana* to *Zoophycos* to *Nereites*—indicate a progressive increase in water depth for the central part of the basin. Forty-five samples from shallow water bioclastic limestones of the *Cruziana* facies yielded 7,000 conodonts, dominated by *Adetognathus* (high population; low diversity). Forty samples from limestones in the deeper *Zoophycos* facies yielded only 1,000 specimens, dominated by *Idiognathus* with some *Adetognathus* species (few individuals; high diversity). From micritic carbonates and siltstones in the deep basin *Nereites* facies, 45 samples produced only a few fragmentary conodonts—excluding some introduced by turbidity currents from shallower environments—(individuals rare to absent). These data demonstrate the usefulness of conodonts in identifying specific depth regimes in ancient basins. Moreover, the virtual absence of conodonts from the deeper regions of the basin, estimated to be about 500–700 m deep, and the marked lateral variation in faunal composition with little mixing indicate that the conodontophorids had a nektobenthic habit.

The early phases of closing of the proto-Atlantic Ocean are, on the Laurentian Plate, (at least in part), first noticeable in western Newfoundland[13].The first indication of the destruction of the continental margin is provided by the upheaval of the carbonate shelf at the end of the early Ordovician, which resulted in karst topography developing on the carbonate bank (St George Formation) situated at the outer edge of the shelf[14] This upheaval cannot be dated with accuracy using fossils extracted from the bank as they are relatively long ranging and many are as yet undescribed. It can, however, be dated with fossils from the deposits of the continental slope as the slope was elevated but never subaerially exposed, and also by fossils from the initial easterly derived clastic material from the klippen.

The middle Cambrian–early Ordovician Cow Head Breccia of western Newfoundland is considered[15] to be a continental slope deposit with clasts derived from the edge of the shelf (St George Formation) and the uppermost part of the continental slope (lower Table Head Formation).

Ordovician conodonts extracted from limestones interbedded with the breccia and from the matrix are typical of the North Atlantic Province[16]. Lower Arenigian deposits are characterised by the genus *Prioniodus* whereas Upper Arenigian deposits are characterised by *Periodon*. These genera are typical of two neighbouring conodontophorid communities, with the former placed further offshore[2].

During middle Arenigian time the environment of deposition of the breccia seems to have become suddenly shallower; this is particularly well displayed in the conodonts which, with practically no overlap, change from typical *Prioniodus* to *Periodon* faunas[17]. This abrupt changeover occurs in the upper part of the *Prioniodus evae* zone, which correlates with the *Didymograpus nitidus* subzone of the British standard graptolite zonation[18]. Graptolites collected immediately to the south of Cow Head from flysch deposits derived from the incoming klippen also indicate the

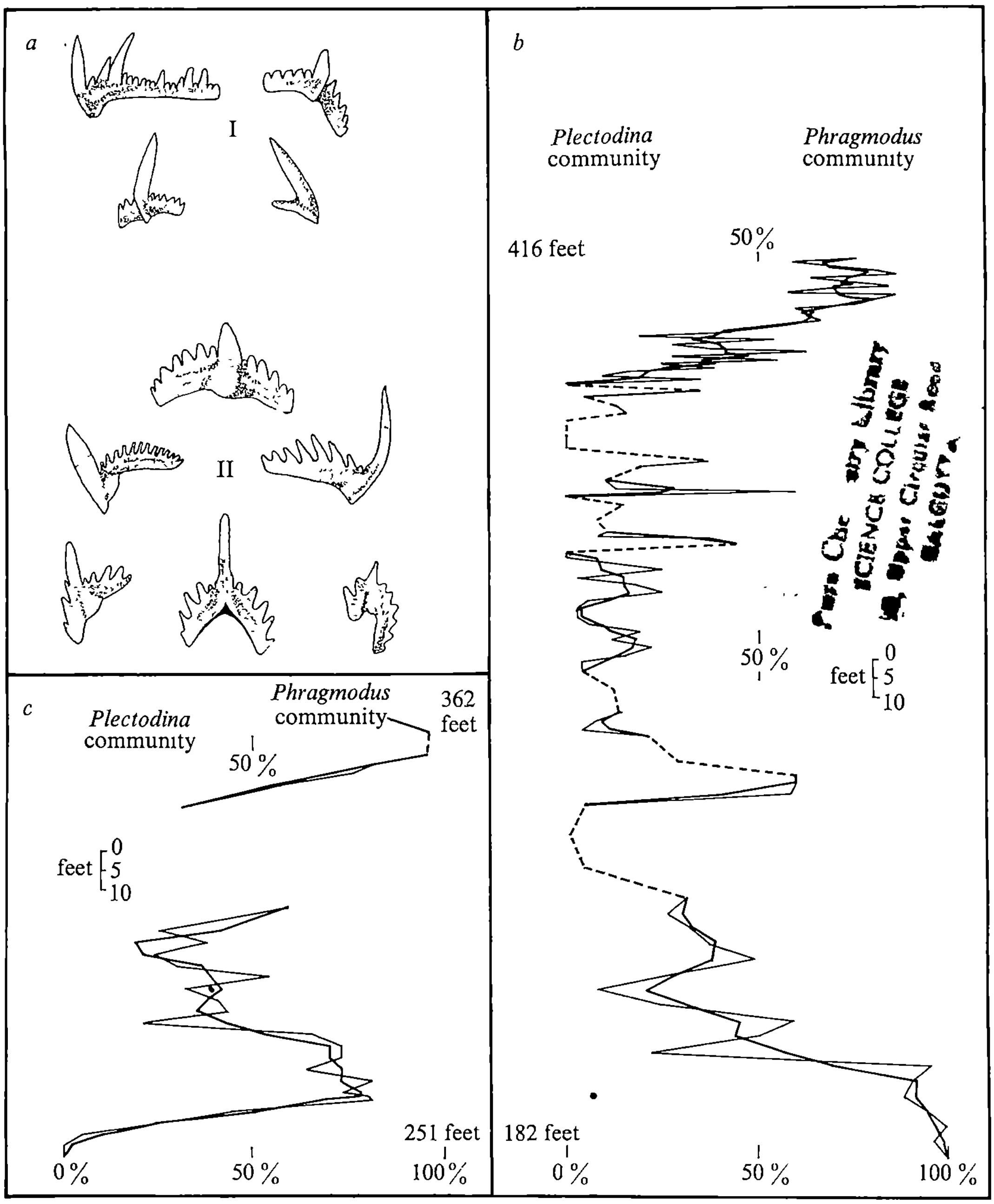


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Fig. 2, *a*: I, Conodont elements of the *Phragmodus* apparatus; II, conodont elements of the *Plectodina* apparatus. *b*, Percentage of *Phragmodus* elements in the total of *Phragmodus* and *Plectodina* elements in a section at Clays Ferry, Madison, County Kentucky (ref. 8, section 60P)—the section is 84 m thick and a total of 128 samples yielded 14,656 conodonts. Fine line, actual percentage; thick line, percentage based on a moving average calculated on every set of three samples starting from the base of the section; dashed line connects numbers where intervening samples contained no conodonts or a number of conodonts considered too small to be statistically significant (20 specimens were arbitrarily chosen as the cutoff limit.) *c*, As for *b* but Section 64S3 of ref 8. Section about 41 m thick, and a total of 32 samples yielded 8,978 conodonts.

D. nitidus subzone. Thus, initial destruction of this ancient continental margin can be dated with considerable precision. Detailed stratigraphic implications of this will be published elsewhere.

Distinctive conodonts are also restricted to extremely shallow water environments. A prime example is provided by the neurodont (fibrous, hyaline) conodonts of the Middle Ordovician. They form a littoral community found in intertidal and lagoonal carbonate environments which probably experienced both high temperatures and salinities[2]. Their virtual absence in deeper shelf facies also suggests a benthic habit[19]. Higher energy environments, developed as local shoal banks or around monadnock islands are characterised by more massive, robust elements. The niches occupied by these neurodonts were largely destroyed with the complete submergence of the North American craton during the late Ordovician, thus explaining their extinction.

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Flatworms afloat

ON March 1, 1975 we sighted from the air, on the surface of the sea near La Paz, Baja California (Mexico), some unusual reddish brown streaks, extending for several hundred metres obliquely to the shore. On the following day we relocated them by ship (RV Dolphin) and found them to consist of high densities of the dinoflagellate *Noctiluca* and of a polyclad turbellarian, provisionally identified as *Stylochoplana sargassicola* (Mertens). Although blooms of *Noctiluca* are not uncommon[1], marine pleustonic flatworms in large numbers are virtually unknown.

Between March 2 and 10, 1975 we made about 20 neuston tows across an area between 22°N and 25°N, and between the western coast of the Baja California peninsula and 110°W. We used a neuston net with fine nylon mesh cod end (202 µm pore size) supported by floats, and sampled swaths of the ocean surface 1 m wide (mean depth 10 cm, depending on wave size) and several hundred metres long. Samples from the brown patches in the La Paz harbour area proved to consist largely of *Noctiluca*, with an admixture of other dinoflagellates; it is probable that the latter were responsible for the brown colour, but that we collected relatively few of them because they were of a size to pass through the pores of the net. We were surprised that samples from all the other brown patches sampled on subsequent days consisted almost exclusively of brown flatworms, associated with more typical pleuston organisms[2] such as *Porpita*, *Physalia*, *Glaucus*, *Ianthina*, *Pleurobrachia*, various smaller copepods, amphipods, decapod megalopa and fish larvae, and, in some tows, an abundance of terrestrial insects. The flatworms were especially numerous in tows taken between 24°20′N and 23°50′N, in some cases clogging the net. A rough calculation indicated that in one area, sampled on March 8, 1975, the surface density of these worms reached 1 m^{-2}. They decreased markedly in abundance south of this zone, and were absent altogether from samples taken south of 23°N.

The animals, of all sizes from 2 to 12 mm long, were beautifully camouflaged among the leaflets of the brown alga, *Sargassum* sp., with which they were often associated. Since the green or brown colorations of certain marine turbellarians, notably species of *Convoluta*, are attributable to the presence of symbiotic algae (respectively, *Platymonas* and *Licmophora* spp. [3,4]), we suspected that the brown granules which constituted the speckles in the body wall of our worms might be symbiotic diatoms or dinoflagellates. They were, however, much too small, too dense and too irregular in shape, and we concluded that they were granules of animal pigment. Unlike dinoflagellates (or *Sargassum*), they did not turn green when denatured by boiling, drying or freezing, and did not yield a green solution when the animals were extracted with acetone, so we dismissed the possibility that they were, or contained, chloroplasts.

The animals displayed three quite distinct modes of movement. (1) They could glide (a few mm s^{-1}), presumably by ciliary movement, on solid substrates (such as seaweed, fish or ctenophores) or on a relatively undisturbed air–water interface. (2) They could swim slowly, in a horizontal position, by undulations of the lateral margins of the anterior two-thirds of the body. (3) Alternatively they could progress much more rapidly by turning on one side and swimming, like a tadpole or small fish, with serpentine undulations, one or two per body length. This mode of progress was presumably noted for *Planaria pellucida* by Mertens[5], who described it as "geschlaengelt" and likened it to the swimming movement of polychaete worms.

They readily settled on most solid substrates (weed, net, bucket and so on) from which they could be dislodged only with difficulty. (They seemed to prefer not to settle on *Porpita*, being perhaps repelled by its stinging nematocysts.) Specimens were rarely observed to be attached by only the posterior end, while the rest of the body swayed freely in the water. They were not seen feeding.

Clusters (3–6 mm diameter) consisting of hundreds of white eggs (100 µm diameter), which may have been laid by these worms, were found attached to the surface of *Sargassum* thalli collected in the same tows. They contained embryos, consisting of a few cells, or early blastulae; their further development was not observed.

Although many marine littoral turbellarians have been described, there have been few reports of truly pelagic species. Hyman[6] mentioned none in her review of marine turbellarians of the Pacific coast of North America. We have been able to trace in the literature several records of pelagic flatworms, but their taxonomy seems to be rather confused. Our specimens closely resemble the polyclad flatworm *Stylochoplana sargassicola* (Mertens)[7] described from the Sargasso Sea by Mertens[5], who found it associated with the seaweed, *Sargassum bacciferum*. It is cryptically coloured, being translucent and speckled with pale yellow or yellowish grey pigment granules. This worm is not confined to the Sargasso Sea, however; it has been found elsewhere in the Atlantic Ocean between 21–35°N and 36–38°W. This species may be synonymous with *Stylochus pelagicus*, described by Mosely[8] from specimens collected along with *Halobates* and other pelagic animals off the coast of Sierra Leone. On both occasions when this worm was collected (August 1873, between 9°21′N 18°25′W and 5°48′N 14°20′W) the sea was highly phosphorescent, presumably, as in our own case, due to large numbers of dinoflagellates such as *Noctiluca*. Apparently *S. sargassicola* has never been reported from the Pacific Ocean before.

Von Graff[7], who referred the nine described pelagic turbellarians to three species (and who also described two new species), recorded another flatworm, *Planaria pellucida* Martens, from the Atlantic as well as the Pacific Ocean. This species, originally described as *P. oceanica* by Charles Darwin[9], was found about 150 miles from Natal, Brazil (5°S 33°W) in February, 1844. Mertens[5] collected his specimens about 500 miles offshore at 7°48′N 23°56′W, where they occurred together with typical pleustonic organisms such as *Porpita*, *Glaucus* and *Physalia*. This species is also somewhat transparent, but the body is whitish yellow, without evident spots. Our specimens seem to be morphologically quite different from *P. pellucida*.

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Production of threitol and sorbitol by an adult insect: association with freezing tolerance

WE report here that in adult tenebrionid beetles, *Upis ceramboides*, an unusual combination of two polyhydric alcohols, sorbitol and threitol, is associated with the ability to tolerate prolonged freezing to at least −50 °C. The occurrence of threitol is of particular interest, since this compound has not previously been found in nature[1].

Following the discovery that immature insects may produce glycerol during diapause[2], polyhydric alcohols, usually glycerol, have been implicated as cryoprotective agents in a variety of overwintering insects[3,4]. Because of its relatively small molecular size, glycerol is able to penetrate most cell membranes, and its ability to prevent freezing damage is generally believed to lie in its colligative properties and its ability to act as a solvent, keeping potentially harmful salts in solution as they are concentrated during ice formation[5]. Although non-penetrating compounds, including sorbitol and mannitol, are reported to have cryoprotective action on isolated cells[5] there are no reports of such a function in whole insects. Sorbitol has been found in the eggs of two insects[6,7] and in the larve of the dipteran *Eurosta solidagenis*[8]. The latter insect is tolerant to freezing but the function of sorbitol is obscured because glycerol is also present. The only other polyol reported to occur in insects is mannitol, which occurs with glycerol in the eggs of the overwintering aphid *Hyalopterus*[9].

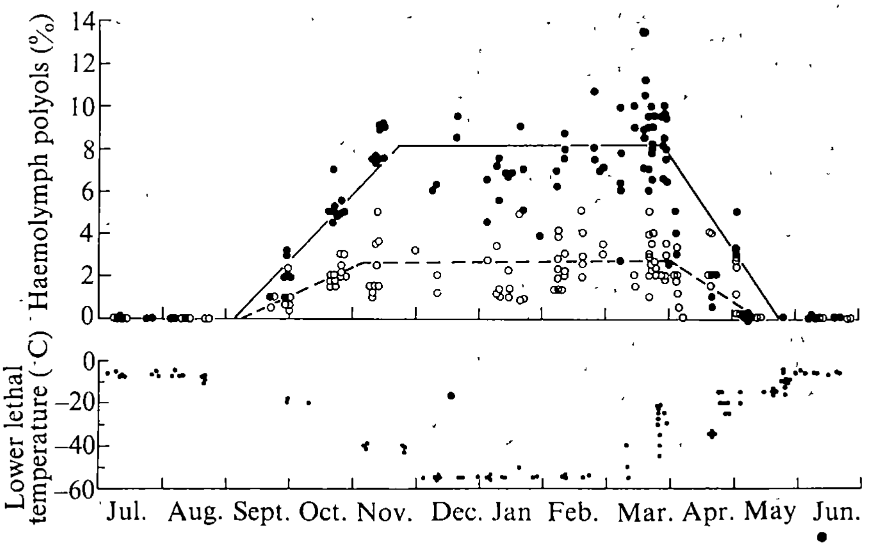
Freezing tolerance in an adult insect was first documented in a carabid beetle, *Pterostichus breviocornis*[10], and is associated with the accumulation of large quantities of glycerol[11]. A more extensive survey of overwintering northern insects has revealed a number of freeze-tolerant species (L. K. M., unpublished), but *Upis ceramboides* is of particular interest because its large size (~ 150 mg) makes analysis of a variety of haemolymph constituents in each individual possible. It overwinters in a very exposed habitat above the snowline, and, as we show here, synthesises large quantities of polyols other than glycerol.

Adult *Upis* may be found throughout the year in interior Alaska and Canada beneath loose bark of aspen, birch, and spruce. During winter, air temperatures in these regions may fall to −50 °C and they often remain below freezing point for many months. Beetles collected during winter and brought into the warm laboratory recover their normal locomotion and frequently begin to feed within several hours. The relative scarcity of *Upis* has made it necessary to accumulate data over a 4-yr period to obtain sufficient seasonal information. In addition to analysis of haemolymph polyols, information was obtained on lower lethal temperatures, supercooling points and other physicochemical characteristics not included here. Because of the temperature lability of insect polyol levels, care was taken to perform all tests less than 1–2 h after specimens were brought into the laboratory. If specimens had to be stored, they were kept outdoors in conditions approximating natural ones.

Supercooling points have been determined using a thermoelectric technique on a large number of specimens and average −6.3 °C, which is quite high for a cold hardy insect and, surprisingly, shows no significant seasonal variation in spite of changing polyol levels Initial tests for polyhydric alcohols using paper chromatography with several solvent systems showed that no glycerol was present but that sizeable amounts of either sorbitol or mannitol, and what was believed to be erythritol, were present during the winter months. Polyol levels drop to undetectable amounts in the summer and build up during the autumn (Fig. 1).

To identify whether it was sorbitol or mannitol that was

Fig. 1 Haemolymph concentrations of threitol (– – –, ○) and sorbitol (——, ●) during the year and their relationship with lower lethal temperatures in *Upis ceramboides*. Data points represent individual values obtained over a 4-yr period from 1970–71 to 1973–74. Polyol levels were determined using paper chromatography with a solvent system of butanol–acetic acid–water (12:3:5 v/v), and periodate followed by borate–starch for detection. Positive identification was by GLC using a 150 cm × 3 mm glass column with 10% SP-2401 on Supelcoport 100/120, 220 °C column temperature. Lower lethal temperatures represent 100% mortality following a 1-h exposure to the final temperature. Cooling rates were 0 3 °C min⁻¹.



being produced by *Upis*, gas-liquid chromatography (GLC) of alditol acetates of the polyhydric alcohols was used[12,13]. It was found that sorbitol is produced, not mannitol, but the compound previously identified as erythritol was found to have a slightly greater retention time than the erythritol standard. On the basis of previous work[14] on the separation of acetylated derivatives of tetritols we concluded that the unknown polyol must be threitol. A sample of threitol was obtained as a standard and compared with *Upis* fat-body extracts and individual and mixed standards of glycerol, sorbitol, and (meso) erythritol using GLC[12,13]. Distinctly separate, well defined peaks were obtained for each polyol in the standards and extracts. The tetraacetate for our D-threitol standard consistently showed identical retention times with the unknown polyol in *Upis* fat-body extracts, and the threitol standard and the polyol in the fat-body extracts invariably had retention times that were 15% longer than the erythritol standard. Although the threitol standard we obtained was D-threitol, the GLC technique cannot distinguish D and L forms on the basis of retention times, so we can only be sure that the polyol in *Upis* is an enantiomer of threitol, and is not erythritol.

The wood-rotting fungus *Armillaria mellea* has been reported to produce threitol on an artificial glucose medium[15], so it was possible that the beetles had been feeding on rotten wood containing *Armillaria*. To test this possibility, a group of *Upis* were deacclimatised at room temperature until their haemolymph polyols dropped to zero The beetles were then cooled in stepwise fashion over a period of weeks and tested at intervals for polyols (Fig. 2). It is evident that the cold reacclimatised beetles were able to produce sorbitol and threitol in response to the artificial cold stress.

On the basis of the correlation between haemolymph polyol levels and freezing tolerance (Fig. 1) it seems that either sorbitol or threitol, or both, have a cryoprotective function in *Upis ceramboides*. This is the best evidence to date that polyols of higher molecular weight than glycerol can serve as natural cryoprotectants. This is the more unusual because it applies to a large, adult insect with its inherently greater tissue complexity and susceptibility to temperature gradients in the body during ice formation. Since threitol contains only one more carbon atom than glycerol, it may be able to penetrate the cells of *Upis*. Indirect evidence for this is provided by the very low cooling rates, 0.3 °C min^{-1} or lower, which are required to prevent freezing damage at temperatures below −30 °C (L. K. Miller, unpublished), and that penetrating cryoprotective agents are typically associated with protection against slow freezing injury[16].

Because of its higher molecular weight, the function of sorbitol in freezing tolerance is more difficult to comprehend, although the present study provides stronger circumstantial evidence for its importance. If sorbitol does act as a non-penetrating cryoprotectant, its very high concentration in *Upis* haemolymph is somewhat of a mystery since non-penetrating agents are generally effective at very low concentrations[4].

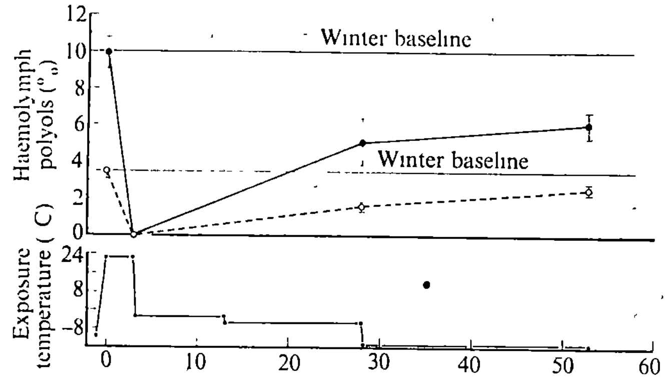


Fig. 2 Changes in haemolymph concentrations of threitol (– – –), and sorbitol (——) at different acclimatisation temperatures. Note the disappearance of polyols after exposing winter-adapted beetles to 23 °C for only 3 d, followed by the more gradual buildup of alcohols during subsequent cold exposure.

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Endogenous rhythm of nerve activity in the housefly eye

WHEN a glass electrode filled with KCl is placed on the eye of a housefly bursts of nervous activity occurring even in darkness may be observed using an oscilloscope. We present evidence that the diel changes in burst frequency represent a true circadian rhythm. The source of these signals obtained from the housefly eye is as yet unknown. Dethier[1] reported two types of electrical activity as recorded externally from the insect eye, the electroretinogram (ERG) and spontaneous nerve firing. The latter activity was related to light intensity and movement in the visual field.

An endogenous circadian rhythm in the rate of nerve firing in darkness from the housefly eye is not inconsistent with current information on insect circadian rhythms as reviewed Brady[2]. Other physiological activities of insects are reported to have at least diel fluctuations. The only previous reports of daily fluctuations in the electrical response of arthropod visual units were both concerned with responses to light. Jahn and Crescitelli[3] suggested a diurnal change in the ERG of a carab beetle, and Aréchiga and Wiersma[4] found a circadian rhythm in the response of the crayfish optic nerve to light.

In our experiments, flies with an active KCl electrode in contact with the corneal surface of the eye were shielded in a light-proof metal box. Neural activity was monitored at hourly intervals on an oscilloscope and the discrete bursts of spikes (Fig. 1) were counted. Recordings from individual flies reared in darkness and handled only in red light could be made over several periods of 24 h or more. The rate of spontaneous nervous activity in the single housefly eye over 24 h is shown in Fig. 2*a*. The rhythm is monophasic with a period of approximately 24 h. The rhythms of individual flies monitored over the same 24 h period showed peaks at random times. The rate of nerve activity during the peak periods was 10–20 times greater than base levels. The period of increased activity lasted 8–12 h.

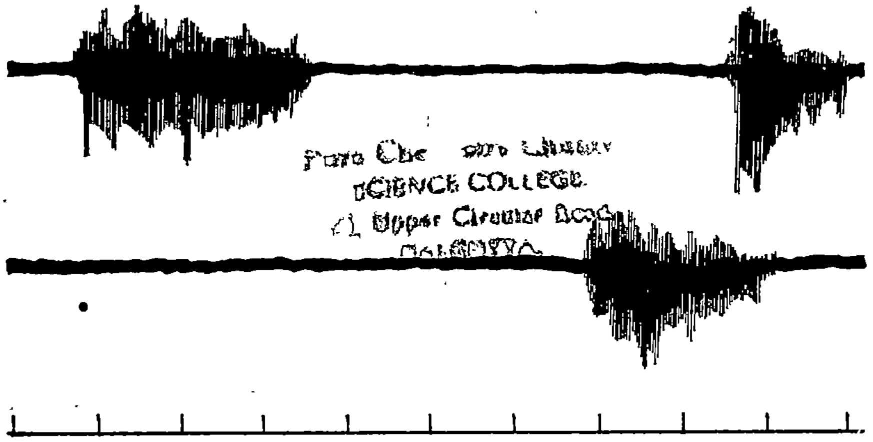

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Fig. 1 Bursts of impulses recorded externally from the housefly eye in darkness (trace speed: 200 ms per division).

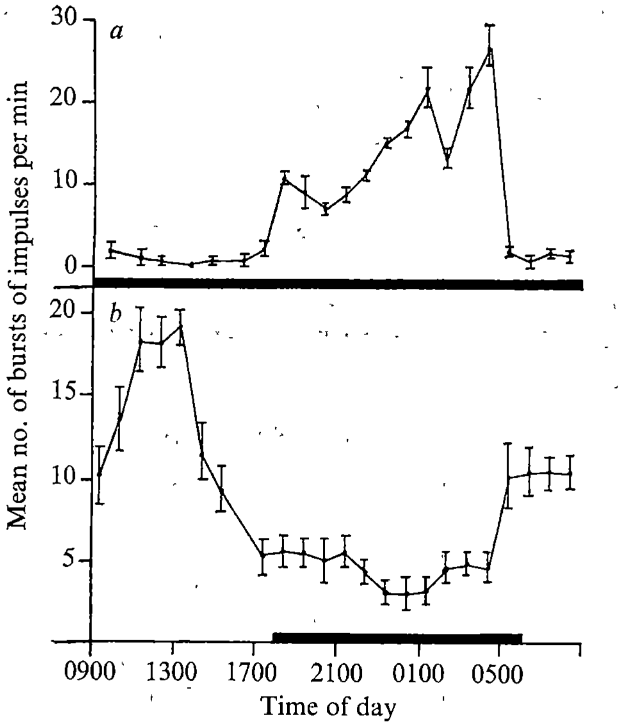
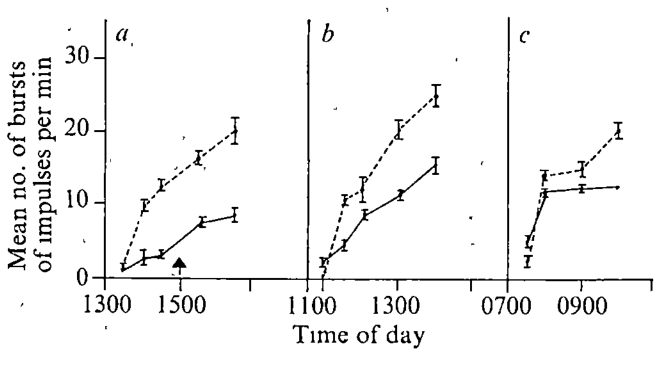


Fig. 2 Variation over 24 h in mean hourly rate of nerve activity in darkness from *a*, single housefly eye; *b*, from groups of houseflies reared and handled in light–dark 12:12, photophase 0600–1800. Bars represent s.e.

Fig. 3 Mean rate of eye nerve activity in darkness, from two houseflies, over the time of the start of the activity peak on three successive days (bars indicate s.e.). Flies reared dark–dark. Nerve activity rhythms were synchronised by an entraining photophase, 1500–2200, the day before day 1 (*a*). On day 1 flies anticipated "dawn" (indicated by arrow) but thereafter the flies displayed a free running period. *b*, Day 2; *c*, day 3.



We also have evidence that the rhythm can be entrained by a photoperiod (Fig. 3) and has a free-running period of 20–22 h. Experiments involving mean hourly responses of groups of flies reared in light–dark 12 : 12 and 16 : 8 artificial light photoperiods indicated that the flies were able to anticipate "lights on" (Fig. 2*b*). Results showed the beginning of the peaks of nervous activity were synchronised to start about half an hour before dawn.

Our experiments are continuing and the details of our findings will be reported elsewhere.

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Recognition of self from non-self in crustaceans

THE immune response of vertebrate species seems initially to involve recognition events between immunogenic molecules and specific lymphocyte receptors. These recognition events subsequently result in amplification steps including, in many cases, increases in cells capable of producing humoral antibodies or participating in cell mediated reactions[1]. Since invertebrates apparently cannot make humoral antibodies[2,3] but can reject allografts[4,5] it seems appropriate to inquire if such animals have receptors capable of discriminating self from non-self. The similarities between antigen receptors and humoral antibodies in vertebrates[6,7] serve as additional reasons for investigating recognition mechanisms in invertebrates. Although sufficient data exist to indicate that the lectin-like agglutinins present in haemolymph can function as opsonins for foreign erythrocytes in certain invertebrates[8], considerations of foreignness of protein molecules are not as definitive. Certain crustaceans, such as crayfish[9] and lobsters[10], can readily clear certain mammalian serum albumins from the circulation but little is known of the specificity of this elimination. We have investigated whether crustaceans possess recognition molecules (referred to as receptors) enabling them to recognise various proteins as foreign and to eliminate these proteins from the circulation. The basic premise was that if such receptors are present, regardless of their location (cell-bound or free), they should be of finite number (and thus saturable) and have some discernable degree of specificity.

Table 1 Distribution of radioactivity in crayfish tissues 2 h after injections of ^{125}I-BSA

	Animal 1			Animal 2		
Tissue	Tissue weight	Counts per 0.1 min per g tissue	Counts per 0.1 min per total tissue	Tissue weight	Counts per 0.1 min per g tissue	Counts per 0.1 min per total tissue
Gills	2.6	51,000	132,600	2.2	50,000	110,000
Heart	0.2	2,500	500	0.5	2,000	1,000
Hepato-pancreas	1.6	2,800	4,480	1.6	500	800
Digestive tract	1.1	1,200	1,320	1.1	1,600	1,760
Green gland	0 12	30,000	3,600	0 3	59,000	17,000
Muscle	5.3	1,800	9,540	4.0	3,000	12,000
Exoskeleton	18.0	100	1,800	19.0	1,000	19,000
			Total 153,800			161,560

*Each animal received 200,000 counts per 0.1 min at $t = 0$ h.

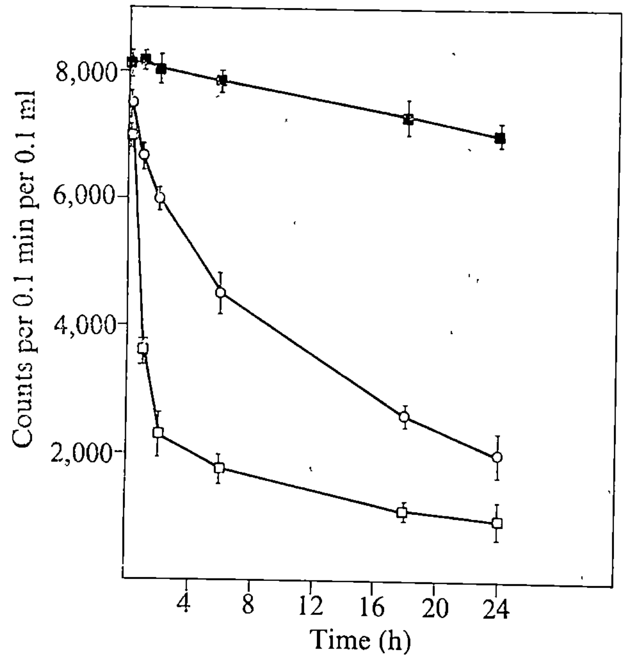

Fig. 1 Levels of radioactivity in the circulating haemolymph of crayfish at various times after injecting ~ 5 μg radioiodinated BSA (□), HGG (○) and crayfish haemocyanin (■). Each experimental point represents the mean of duplicate counts on three animals The vertical bars indicate the limits of ± 1 s d.

The initial experiments were aimed at determining if the crayfish, *Procambarus clarkii*, could clear different proteins from the circulation. The basic protocol involved injecting into the heart of 25–30 g animals maintained at 23 °C small amounts (~ 5 μg in 0.1 ml) of ^{125}I-labelled proteins[11]. At various times samples of haemolymph were removed and assayed for radioactivity. Control (self) proteins included crayfish haemocyanin and a 5S crayfish protein of unknown function (purified by gel filtration from clotted haemolymph). Each of these self proteins, as depicted for crayfish haemocyanin in Fig. 1, seemed to equilibrate within 10 min (with a volume approximating 18% of the animal's weight) and to exhibit almost no elimination (<20%) during 24 h. On the other hand (Fig. 1), if similar amounts of either iodinated bovine serum albumin (BSA) or human gamma globulin (HGG) were injected, relatively rapid clearance was observed. In the case of BSA about 80% of the radioactivity was eliminated within 2 h; elimination of HGG was somewhat slower. Two additional foreign proteins studied included keyhole lympet haemocyanin (KLH) and egg white lysozyme; the former was eliminated somewhat more slowly than HGG and the latter at about the same rate as BSA.

The next step was to determine the fate of these cleared proteins. First, several crayfish injected with BSA or HGG, maintained in small beakers containing ~ 5 ml water, did not excrete significant amounts of radioactivity during 24 h and thus the injected proteins were still within the animal. Second, as Table 1 shows, dissection of several animals 2 h after injection of BSA indicated that most of the cleared radioactivity was localised in the gills (>60% of that recovered). Further, the specific activity (counts per gram of tissue) of the green gland, an excretory organ, was also quite high. The relatively small amount of tissue present, however, suggests that this gland does not have a major role in the initial clearance.

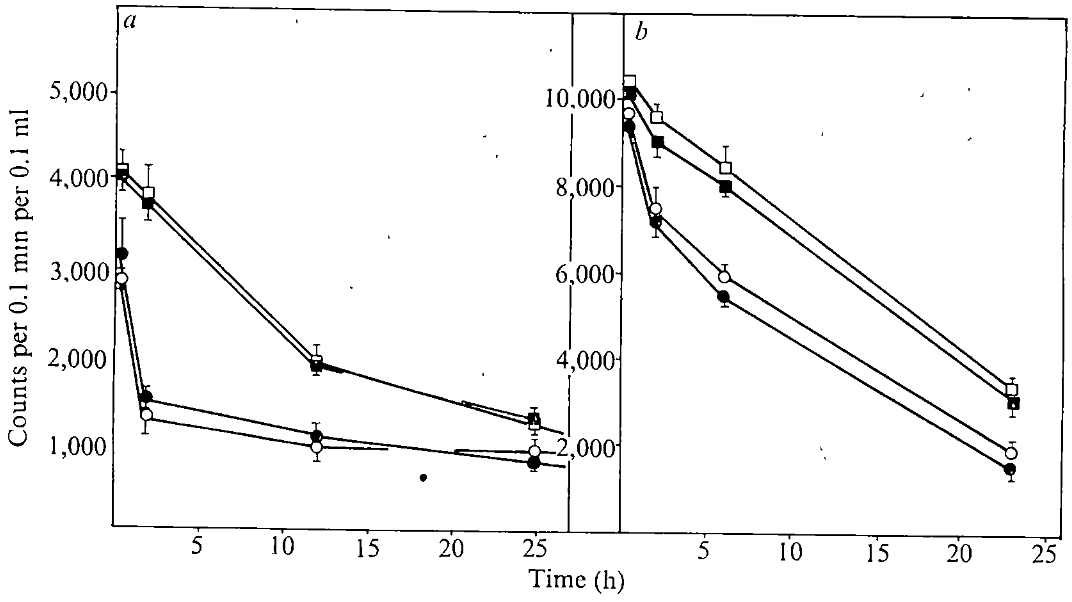

Fig. 2 Levels of radioactivity in the circulating haemolymph of crayfish at various times after injecting mixtures consisting of ~ 5 μg of radioiodinated proteins and 5 mg unlabelled proteins. Each experimental point represents the mean of duplicate counts on three animals. The vertical bars indicate the limits of ± 1 s.d. *a*, ^{125}I-BSA plus: ●, nothing; ○, bovine gamma globulin; □, BSA, ■, human serum albumin. *b*, ^{125}I-HGG plus; □, HGG; ○, BSA; ■, BGG; ●, nothing.

Since the radioactivity injected on foreign protein was not cleared totally, trichloroacetic acid precipitation of the haemolymph was used to show if protein modification had occurred. The data obtained indicated that the 10-min BSA samples were ~ 95% precipitable, the 2-h samples were 90% precipitable, the 6-h samples ~ 70% and the 24-h samples only 50% precipitable. A similar pattern was obtained with HGG. On the other hand, the labelled circulating crayfish haemocyanin remained >95% precipitable for 24 h. It therefore seems likely that the clearance curve for BSA shown in Fig. 1 represents two reactions—an initial concentration of the protein in the gills followed by degradation to small peptides which are released back into the circulation.

These experiments indicate that crayfish can readily discern self from non-self by clearing various foreign proteins from the circulation and concentrating them in the gills. We then investigated whether this recognition involved saturable receptors of reasonable specificity. We injected each animal with a mixture of ~ 5 μg ^{125}I-labelled protein and ~ 5 mg unlabelled protein. As Fig. 2*a* shows, excess unlabelled BSA seemed to retard the elimination of radioactive BSA whereas unlabelled BGG had no effect. Similarly KLH (not shown) had no effect on the elimination of ^{125}I-BSA whereas other serum albumins (human, shown in Fig. 2, and horse and rabbit, not shown), were as effective in retarding clearance as BSA when 5 mg was used. Alternatively, as Fig. 2*b*, shows both HGG and BGG slowed down the initial elimination of HGG whereas BSA and KLH (not shown) did not. Additional experiments (not shown) indicated that unlabelled KLH blocked the initial clearance of ^{125}I-KLH whereas BGG and BSA did not.

In conclusion the data reported here support the hypothesis that crayfish have naturally occurring receptors, or recognition molecules, for at least three groups of foreign proteins (albumins, gamma globulins and haemocyanins). Although it is premature to generalise about the signficance and distribution of such putative receptors in other invertebrates, initial clearance and inhibition studies indicate their likely presence in another crustacean, the blue crab (McCumber and L.W.C., unpublished results) and in a mollusc, the chiton (Lafferty, presentation at Immunologic Phylogeny Symposium, Hawaii, June 1975). Optimistically, further pursuit of these studies might very well result "in the isolation of the primitive building block for vertebrate immunoglobulins"[12].

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Existence of a β-ionone ring-binding site in the rhodopsin molecule

Rhodopsin is a carotenoid-protein, in which the 11-*cis* retinal chromophore, is coupled to the colourless protein, opsin. The linkage between them is a protonated Schiff base between the aldehyde group of 11-*cis* retinal and an ε-amino group of L-lysine in opsin[1-4].

We report here the competitive inhibition of the combination of 11-*cis* retinal with opsin by β-ionone and discuss from this viewpoint the structure of the retinal-binding site of the opsin molecule.

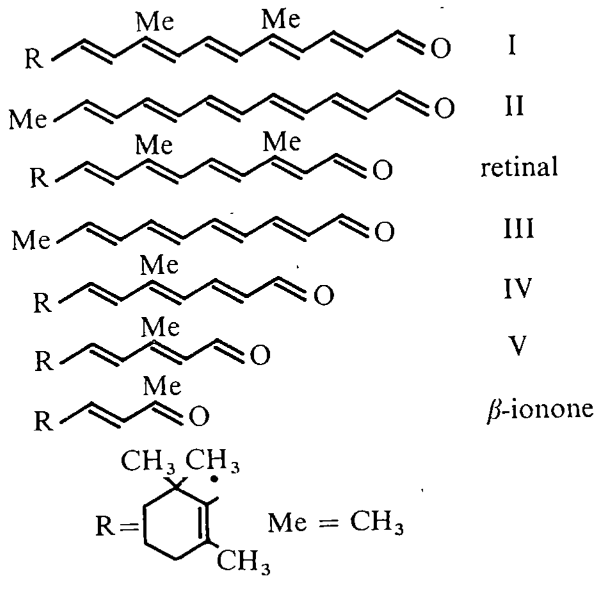
The existence of secondary interactions between 11-*cis* retinal and opsin has been suggested by many workers, without direct evidence. One possible way to study such interactions is to use 11-*cis* retinal analogues. Blatz *et al.*[5] reported that the 11-*cis* retinal analogues shown in Fig. 1 do not form photosensitive pigments when incubated with cattle opsin. From their result one may conclude: (1) The length of the side chain of an 11-*cis* retinal analogue is a decisive factor in coupling with opsin. Retinal analogues having longer or shorter side chains than 11-*cis* retinal (compounds I, II, IV, and V) do not form photosensitive pigments. It may be said that opsin can discriminate between the lengths of the side chain of the retinal analogues. (2) A ring structure of the analogue seems essential for the regeneration of rhodopsin. Some analogues having a methyl group instead of a β-ionone ring (compounds II and III) fail to form a pigment.

We have now examined the possibility that the β-ionone ring of 11-*cis* retinal may interact with opsin through hydrophobic bonds; the regeneration of rhodopsin may begin with the formation of such bonds, and end in the formation of the protonated Schiff base linkage. Only when the aldehyde group of an analogue, already bound to opsin through the β-ionone ring, comes sterically near to the ε-amino group of an L-lysine residue at the 11-*cis* retinal-binding site in opsin (we call this the 'active L-lysine'), can the Schiff base be formed.

If that is so, we can expect that an analogue having a β-ionone ring, but a shorter side chain than retinal (compounds IV, V and β-ionone), may readily compete with 11-*cis* retinal in coupling with the site that anchors the ring structure.

Cattle opsin was prepared from ~ 50 light-adapted retinae by the usual methods[6]. The opsin was extracted with 2% digitonin in M/15 phosphate buffer (*p*H 6.5). Regeneration of rhodopsin occurred at 25 °C in an absorption cell of 1 cm light path placed in a recording spectrophotometer, and the absorbance at 498 nm was recorded as shown in Fig. 2*a*. Curve 1 shows the regeneration of rhodopsin from 11-*cis* retinal (final concentration 1.91×10^{-5} M) and opsin (final concentration 2.76×10^{-6} M). Addition of β-ionone (final concentration 3.68×10^{-4} M) to the reaction mixture slowed the regeneration of rhodopsin (curve 2). Both curves reach almost the same final

Fig. 1 Retinal and its analogues. None of these analogues form a photosensitive pigment on incubation with cattle opsin (Blatz *et al.*[5]).



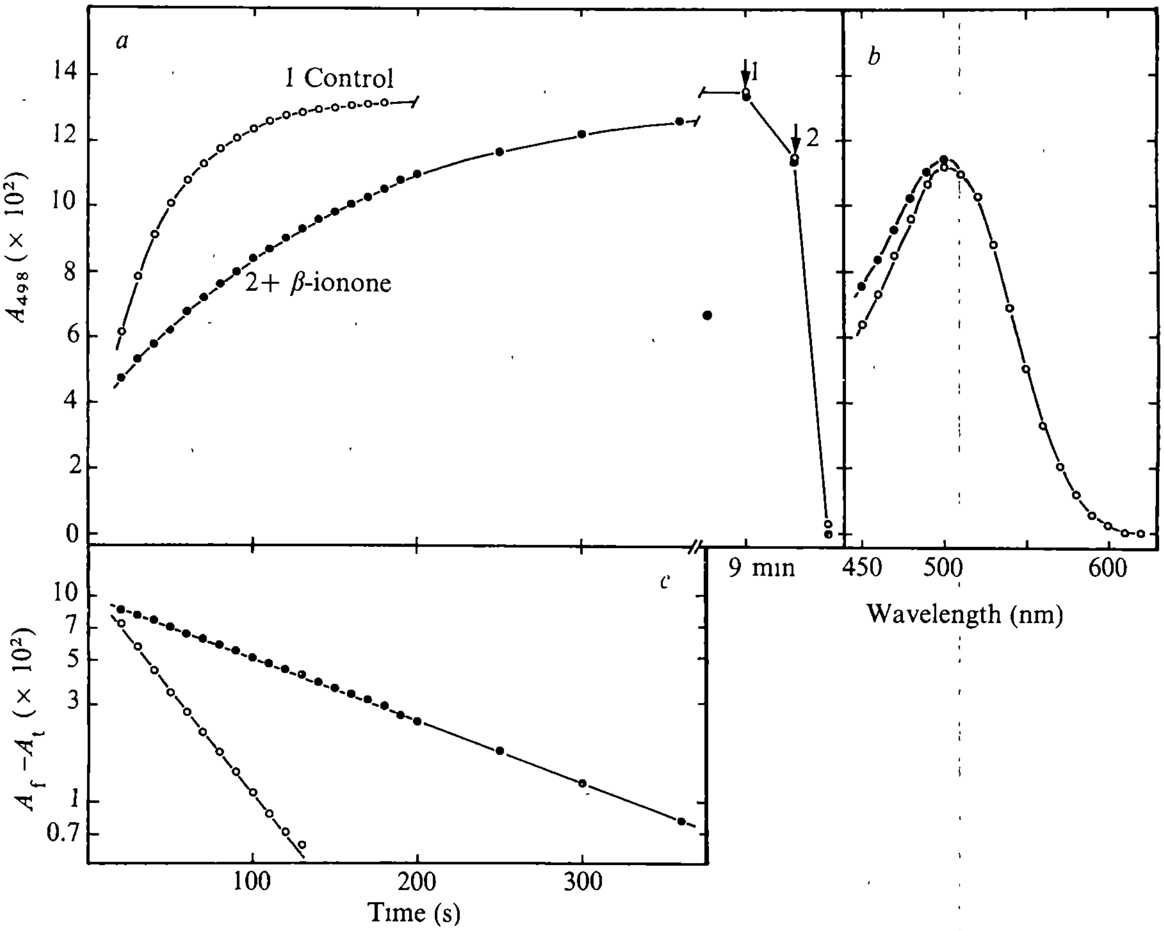


Fig. 2 Inhibition by β-ionone of the combination of 11-*cis* retinal with cattle opsin. *a*, Time course of the regeneration of rhodopsin. The reaction was performed at 25 °C in a cell (light path: 1 cm), containing 0 1 ml cattle opsin extracted with 2% digitonin (M/15 phosphate buffer *p*H 6.5), 2.0 ml M/15 phosphate buffer (*p*H 6.5) and 0.1 ml β-ionone (8.13×10^{-3} M) dissolved in 1% Brij 58 in M/15 phosphate buffer *p*H 6.5. In the control, 0.1 ml 1% Brij 58 was added instead of the β-ionone solution The reaction was started by adding 10 μl 4.23×10^{-3} M 11-*cis* retinal in ethanol. The absorbance change at 498 nm was measured with a Hitachi 323 recording spectrophotometer. After the completion of the reaction 30 μl 1 M NH_2OH were added (arrow 1). Three minutes later, the preparations were irradiated with white light to bleach the regenerated rhodopsin (arrow 2). The concentrations of 11-*cis* retinal (ε_m: 24,900 in ethanol), cattle rhodopsin (ε_m: 40,600) and β-ionone (ε_m: 8,700 in ethanol) were determined spectrophotometrically. *b*, The difference spectra before and after bleaching of the regenerated rhodopsin. *c*, Pseudo first-order plots of Fig. 2*a*, where A_f is the final absorbance at 498 nm when the regeneration was completed and A_t is the absorbance at 498 nm at time, *t*.

levels of absorbance, indicating that no denaturation of opsin occurred in the experiment. After the completion of the regeneration reaction, hydroxylamine (final concentration 1.34×10^{-2}M) was added to both preparations (arrow 1 in Fig. 2*a*), and then the preparations were irradiated by white light to bleach the regenerated rhodopsin (arrow 2) completely. Figure 2*b* shows the difference spectra before and after bleaching. Because of the high absorbance caused by excess 11-*cis* retinal the spectra could not be measured correctly below 450 nm. Both difference spectra coincided reasonably with each other.

In this experiment both regeneration curves can be represented by pseudo first-order kinetics as shown in Fig. 2*c*, because there was 6.9 times as much 11-*cis* retinal as opsin. The pseudo first-order rate constants are 2.90×10^{-2} s^{-1} for regeneration in the absence and 7.45×10^{-3} s^{-1} in the presence of β-ionone. If one divides the pseudo first-order rate constants by the retinal concentration (1.91×10^{-5} M), the second-order rate constant of the regeneration in the absence or presence of β-ionone (3.68×10^{-4} M) is 1.52×10^3 mol^{-1} s^{-1} or 3.94×10^2 mol^{-1} s^{-1} respectively.

The regeneration rate constant of cattle rhodopsin was reported by Wald and Brown[7] to be 43.3 mol^{-1} s^{-1} (22.5 °C, *p*H 7.0). The discrepancy between their rate constant and ours may be explained by the difference in digitonin concentration of the preparations—theirs being 2% and ours about 0.09%, and also by the presence of 0.045% Brij 58 in our experiments. We have found that the lower the concentration of digitonin, the greater the observed regeneration rate constant of cattle rhodopsin, and that the presence of Brij 58 may slightly accelerate the regeneration (H.M. and T.Y., unpublished).

The possibility that the keto group of β-ionone may form a Schiff base with the active L-lysine of opsin was excluded by other experiments. Incubation of cattle opsin with β-ionone formed no pigment displaying absorbance between 300 and 700 nm. In addition, β-ionol, which was derived from β-ionone by reduction with sodium borohydride, also inhibits the regeneration of rhodopsin to the same extent as β-ionone. Thus, the inhibition by β-ionone must be attributed to its binding to some hydrophobic region of the retinal-binding site in opsin rather than to the active L-lysine. We call the hydrophobic region here the 'β-ionone ring-binding site'.

The binding of β-ionone to the β-ionone ring-binding site in the opsin molecule is faster than the formation of the Schiff base, because the addition of β-ionone at the beginning of the reaction does not change the regeneration kinetics of rhodopsin compared with when β-ionone is preincubated with opsin (H. M. and T. Y., unpublished).

One can write the mechanism as follows

$$\text{opsin} + \beta\text{-ionone} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \beta\text{-ionone–opsin complex}$$

$$\text{opsin} + 11\text{-}cis\text{ retinal} \xrightarrow{k_3} \text{rhodopsin}$$

where the rate constant of the formation of the β-ionone–opsin complex, k_1, is much greater than that of rhodopsin formation,

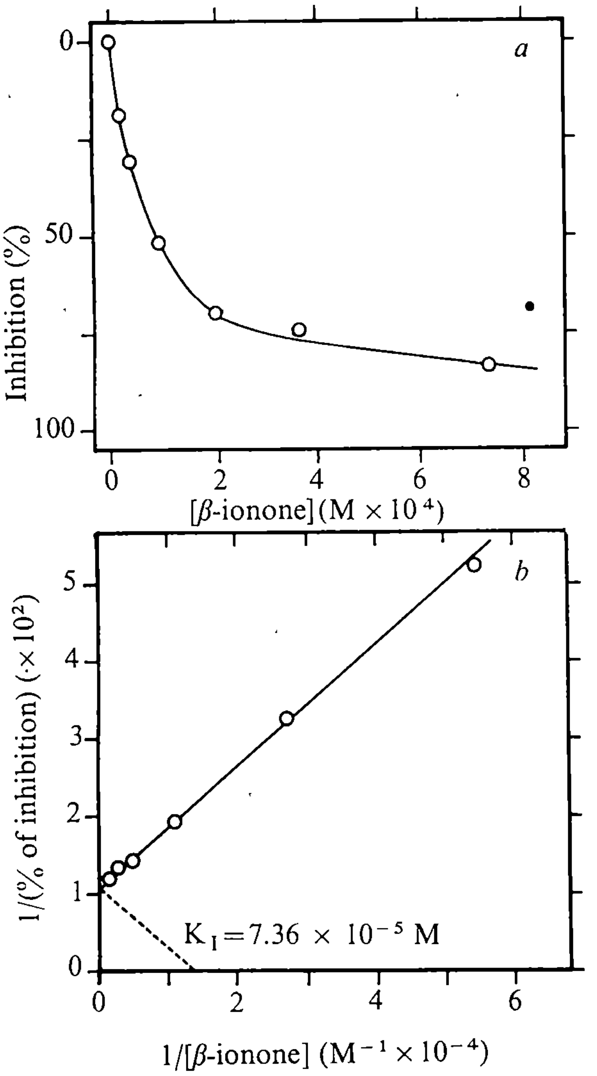


Fig. 3 Dependency of the inhibition on the concentration of β-ionone. *a*, Relationship between percentage inhibition and the concentration of β-ionone. The same reaction mixture were used as in Fig. 2 except that the volume of 1% Brij 58 solution was changed from 10 μl to 0.2 ml. The total volume of the reaction mixture was adjusted to constant volume (2.2 ml) by addition or subtraction of M/15 phosphate buffer (*p*H 6.5). The percentage inhibition was calculated from the ratio of the observed pseudo first-order rate constants between the inhibited and the control reactions. *b*, Double reciprocal plot of Fig. 3*a*.

k_3. So, one can determine the inhibition constant (K_I),

$$K_I = \frac{[\text{opsin}]\,[\beta\text{-ionone}]}{[\beta\text{-ionone–opsin}]} = \frac{k_2}{k_1}$$

by determining the relationship between the percentage of inhibition and the concentration of β-ionone (Fig. 3*a*) after measuring the saturation curve of the inhibitor, β-ionone. The curve of Fig. 3*a* has a hyperbolic shape and is described by the equation

$$\text{percentage of inhibition} = \frac{100\,[\beta\text{-ionone}]}{K_I + [\beta\text{-ionone}]}$$

If one plots (percentage of inhibition)$^{-1}$ against (β-ionone)$^{-1}$, a straight line Lineweaver–Burk plot can be obtained as shown in Fig. 3*b*. The cross-over points at the abscissa or the ordinate show the K_I^{-1} or (maximum inhibition)$^{-1}$ respectively. The inhibition constant, K_I, for β-ionone is calculated to be 7.36×10^{-5} M and the maximum inhibition almost 100%.

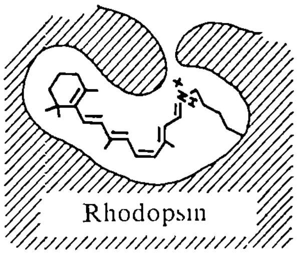


Fig. 4 A schematic representation of the structure of rhodopsin, in which the 11-*cis* retinal chromophore is covered by opsin (dashed area).

Thus, the inhibition by β-ionone is competitive (not partially competitive) in the sense that β-ionone occupies the retinal-binding site so exclusively that the β-ionone–opsin complex is completely inactive in the regeneration reaction. The standard free energy change, ΔG^0, of the formation of the β-ionone–opsin complex, calculated from the equation, $\Delta G^0 = -RT \ln (1/K_I)$, was -5.6 kcalorie mol^{-1}.

We illustrate a hypothetical configuration of rhodopsin in Fig. 4. The chromophore in the figure is drawn as 6-*s*-*cis*, 11-*cis*, 12-*s*-*cis* according to the recent X-ray analysis of 11-*cis* retinal[8]. The β-ionone ring of 11-*cis* retinal is postulated to be bound to the β-ionone ring-binding site in opsin. The fact that rhodopsin is not attacked by hydroxylamine[9] and sodium borohydride[10] (which do attack chicken iodopsin[9,11]) indicates that the Schiff base linkage may be covered by opsin. The polyene side chain of the retinal chromophore is also covered, because lipoxidase does not oxidise the chromophore of cattle[12] and chicken[11] rhodopsin.

According to our model, only those retinal analogues which have the same length of side chain and structure of the β-ionone ring as retinal (as, for example, 9-desmethylretinal, 13-desmethylretinal and 14-methylretinal) are expected to form photosensitive pigments by binding with opsin. This, in fact, has been shown to be the case[5,13,15,16].

The pocket of the β-ionone ring-binding site is thought to have a relatively low stereospecificity, since α-ionone also inhibits the regeneration of rhodopsin competitively (H. M. and T. Y., unpublished). Also, 5-vitaldehyde[14], which has a five-membered ring in place of the β-ionone ring of retinal, and other analogues of retinal which have slightly modified β-ionone rings such as 5,6-dihydroretinal[5,15], 4-oxoretinal[17], 5,6-monoepoxyretinal[18], 3-dehydroretinal[19], α-retinal[20] and 5,6-epoxy, 3-dehydroretinal[21], form photosensitive pigments.

Wald[22] pointed out that the retinal chromophore in visual pigments can fit in a pocket of the surface structure of opsin in two possible ways—by weak bonding through hydrogen bonds or van der Waals' forces, and so-called caging or clathrate formation, in which the chromophore is simply surrounded by opsin without any interacting forces. The low degree of stereospecificity of opsin to the ring structure of the chromophore may support the latter mechanism. To confirm this viewpoint, more detailed kinetic studies on the inhibition of rhodopsin synthesis by β-ionone and other retinal analogues are in progress in our laboratory.

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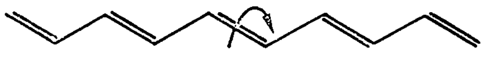
Conversion of a photon to an electrical signal by sudden polarisation in the N-retinylidene visual chromophore

ONE of the major unresolved problems in the chemistry of vision remains the detailed mechanism by which absorption of a photon by rhodopsin can ultimately change the permeability of the rod plasma membrane to Na$^+$ ions[1]. We demonstrate here that excitation to the lowest singly-excited π,π^* singlet state of a realistic model of the visual chromophore triggers a sudden, short-lived, electrical signal in which charge moves from one end of the molecule to the other. We suggest that this electrical signal is the crucial, primary event in the overall mechanism leading to vision.

Diradical intermediates have four chemically important electronic states[2], the properties of which form an integral part of the theory of photochemical reactions[2-5]. We can illustrate the nature of these four states on the orthogonal di-(all-*trans*) pentadienyl diradical, formed by a 90° twist about the central C–C bond of all-*trans* decapentaene



The two lowest states are a (triplet, singlet) pair of diradical states ^{3}D and ^{1}D, and above are two singlet zwitterionic states Z_1 and Z_2 (Fig. 1). These are correctly represented by out-of-phase (−) and in-phase (+) combinations of the two ionic resonance structures.

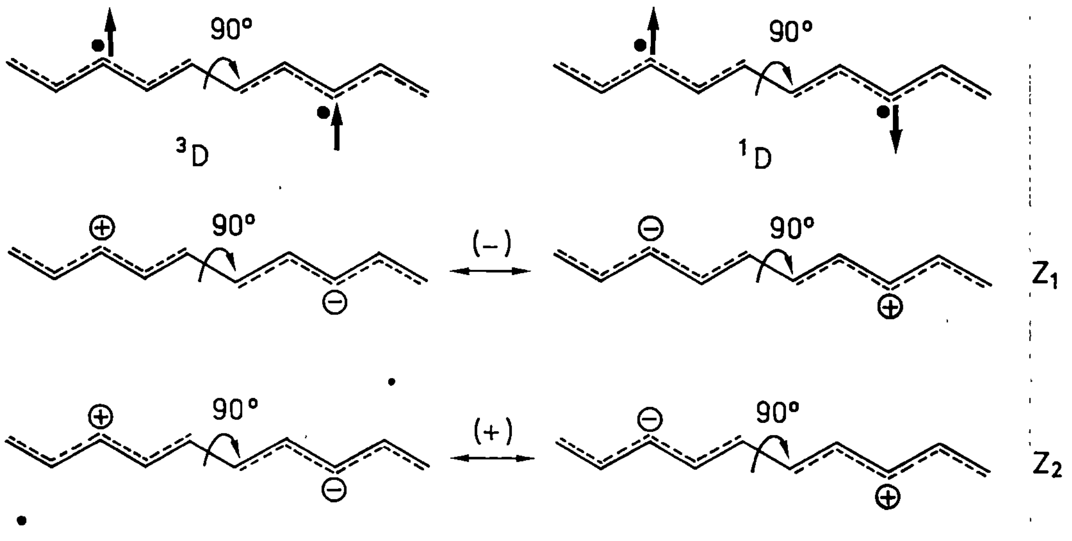
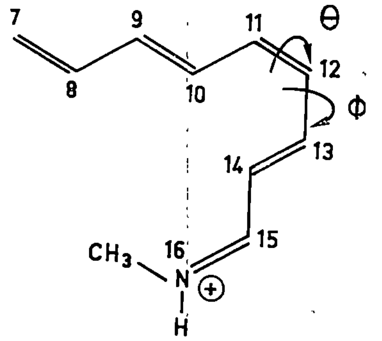


Fig. 1 The four low-lying electronic states of di-(all *trans*)-pentadienyl radical.

It has been shown[6], in confirmation of an earlier prediction[7], that the lowest excited Z_1 zwitterionic states of organic diradicals are strongly polarised if a slight dissymmetry exists between the two odd-electron sites. A sudden, large separation of charge occurs. This 'sudden polarisation effect'[6] can be introduced by a molecular distortion, substitution, or better still by strong pertubations such as the replacement of a carbon atom by a heteroatom. For instance, we would expect the Z_1 excited states of the Schiff base and the protonated Schiff base of dipentadienyl to be strongly polarised (Fig. 2). In both systems the negative charge will flow into the fragment which carries the nitrogen atom. In the Schiff base, the nitrogen will carry most of the negative charge, whereas in the protonated Schiff base the incoming negative charge cancels the positive charge on the nitrogen atom, leaving a now neutral amino group, and a second, newly positively charged, pentadienyl moiety. As the primary photoprocess in rhodopsin is known[1] to involve the isomerisation of the 11-*cis* N-retinylidene chromophore—possibly in the 12-*s-cis* conformation[8] and most probably as a protonated Schiff base[9]—we have investigated the charge distribution in the excited singlet state of the 11-*orthogonal* protonated Schiff base of the retinal skeleton, as well as of the Schiff base itself.

As a realistic model of the chromophore we used molecule I (nonatetraenylidene-methyliminium ion), initially in the 11-*cis*, 12-*s-cis* conformation (for convenience, we number the atoms as in the full retinylidene skeleton). The C5=C6 bond of the cyclohexene ring, which is out of conjugation with the 7–11



I

all-*trans* portion[8,10], was neglected because it would extend the size of I beyond the capacity of our program. The technique for calculating the energies and charge distributions is described elsewhere[3,4]. The basic program is the *ab initio* Gaussian 70

Table 1 Energies and charge distributions in 11-twisted, 12-*s*-*cis* protonated Schiff base I

Twist angle	0°	30°	60°	80°	90°	
Relative energies (eV)						
Ground singlet	0.	−0.23	0.73	1.11	1.18	(^{1}D)
Excited singlet	4.66	4.57	4.04	3.48	3.39	(Z_1)
Net charge in 7–11 fragment:						
Ground singlet	+0.17	+0.19	+0.23	+0.23	+0.23	(^{1}D)
Excited singlet	+0.41	+0.40	+0.55	+0.98	+1.11	(Z_1)
Net charge in 12–16 fragment:						
Ground singlet	+0.83	+0.81	+0.77	+0.77	+0.77	(^{1}D)
Excited singlet	+0.59	+0.60	+0.45	+0.02	−0.11	(Z_1)

Bond lengths: all CH = 1.08 Å, except CH = 1.09 Å in CH_3. At 0° and 30°, C=C = 1.34 Å, C–C = 1.47 Å. For 60°, 80° and 90°, all C–C = 1.40 Å except 11–12 = 1.50 Å. Finally, C=N = 1.31 Å, NH = 1.02 Å. The open-shell Hamiltonian is used for the excited singlet throughout and for the ground singlet from 60–90°. The closed-shell Hamiltonian is used for the ground singlet at 0 and 30°.

program (W. Hehre, personal communication), using a minimal basis set of orbitals and completed by a three-by-three configuration interaction treatment for the proper description of diradicals. Both closed-shell and restricted open-shell Hamiltonians can be used to calculate the energy.

We have calculated energies and charge distributions of the ground and lowest singly-excited π,π^* singlet states of I for various values of the 11–12 twist angle θ (Table 1). We used the experimental[10] torsional angle φ about the 12–13 bond at $\theta = 0°$, and interpolated linearly from $\varphi = 39°$ to $\varphi = 0°$ as θ increases from 0–90°. Table 1 shows that, contrary to the nearly constant charge distribution in the ground ^{1}D diradical state, there is a sharp polarisation of the excited π,π^* state as the twist angle reaches 80°. The positive charge, which is on the nitrogen grouping in the coplanar ground state, shifts somewhat towards the 7–11 moiety in the vertically-excited state, which has considerable intramolecular charge transfer characteristics. The greatest change occurs, however, as this excited singlet molecule twists from 60° to the fully *orthogonal* conformation. All the positive charge flows into the 7–11 pentadienyl fragment, with even a slight negative residual charge left on the Schiff base site (Fig. 3).

The positive charge in excited Z_1 is evenly distributed over atoms 7, 9 and 11, where the amplitude of the non-bonding molecular orbital of the 7–11 pentadienyl fragment is large. This leads to the migration, on excitation, of the positive charge from atom 16 to an average position at atom 9, that is, over a length of seven bonds. The net effect is an extremely large electrical signal. We can estimate the change in dipole from starting molecule to Z_1 by assuming a negative counterion fixed in the vicinity of the initially charged nitrogen atom. Then the initial dipole moment $\mu \approx 0$ and the change in dipole moment $\Delta\mu$ on excitation and twist to the 11-*orthogonal* 12-*s*-*cis* form is given by μ (Z_1). Since atoms 9 and 16 are separated by ~ 6.8 Å in this conformation, $\Delta\mu \approx 33$ Debyes. We have verified that the charge polarisation occurs also in the 12-*s*-*trans* form. In this case, however, the C9–N16 distance in the *orthogonal* conformer is ~ 8.3 Å and the concomitant dipole moment change $\Delta\mu \approx 40$ Debyes.

The vertical excitation energy calculated for I is too high (4.66 eV compared with the experimental value of 2.75 eV, ref. 11). Single-configuration, minimal basis calculations are notoriously poor for vertical π,π^* singlet-state excitation energies[12]. The restricted open-shell method used here, however, is particularly appropriate for describing the 90°-twisted molecule, as the two 'non-bonding' π orbitals Ψ_5 and Ψ_6 are then well separated from the remaining π manifold. The three-by-three configuration interaction is a further guarantee that the four lowest-lying states are correctly described[2]. In this respect the calculated barrier to isomerisation in the ground singlet state (27.2 kcalorie mol^{-1}) agrees nicely with experimental results (25.5 kcalorie mol^{-1}, ref. 13). Finally, the deep calculated minimum (1.27 eV) in the excited twisted singlet is in contradiction with the previous PPP pure π-electron calculations[14,15]. The downwards slope may be emphasised by the exaggerated vertical excitation energy, but the qualitative behaviour seems reasonable since the twisting—just as in ethylene—enables a π^* orbital, which is 11–12 antibonding, to become non-bonding.

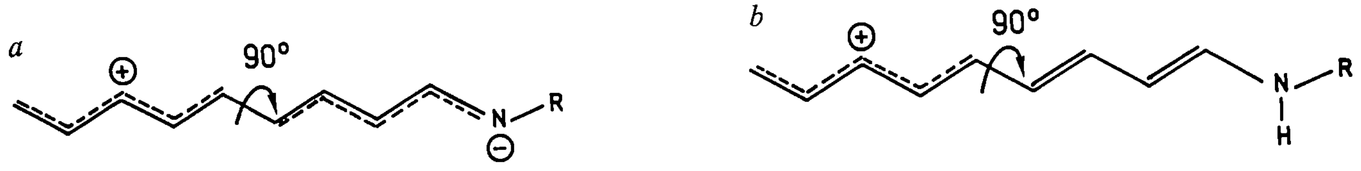
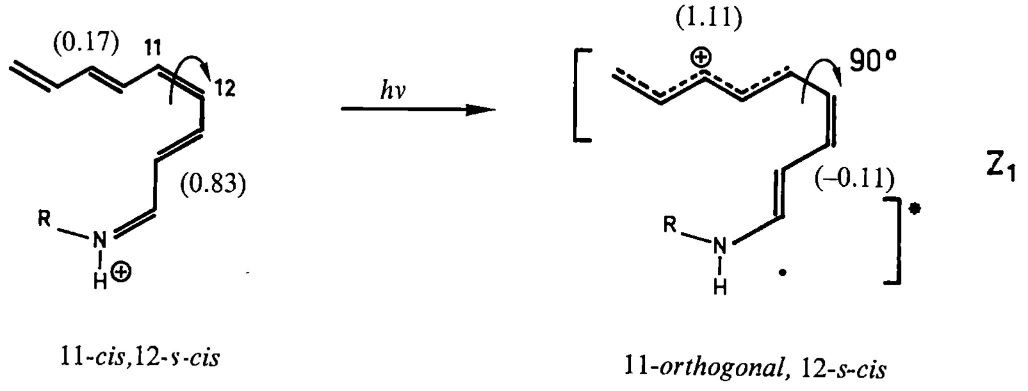


Fig. 2 Expected polarisation of excited Z_1 states in unsymmetrical polyenes: *a*, Schiff base; *b*, protonated Schiff base.

Fig. 3 Excitation and partial isomerisation of the protonated Schiff base (net charges in parentheses).



The previous calculations, with only single excitations[14,15], possibly suffer from an inappropriate description of the wavefunction for Z_1, which involves a double excitation $(\lambda\Psi_5{}^2-\mu\Psi_6{}^2)$.

Clearly, the complete retinal system was not included in the calculation and no external effect was considered. Although we have not been able to investigate the skeleton containing the additional C5–C6 and C6–C7 bonds, or cyclohexene ring, we feel that the qualitative outcome must certainly be the same. Within the ion pair created in the twisted Z_1 state the negative charge must inexorably be attracted by the pre-existing positive charge on the Schiff base site. We have verified that the sudden polarisation effect also occurs in the unprotonated Schiff base of I, where it is accompanied by a significant increase in the basicity of the nitrogen atom. If rhodopsin were to involve a weak $N\cdots H$ bond (ref. 16 and M. Ottolenghi, personal communication), the polarisation effect could trigger a displacement of the proton toward the nitrogen atom (in I itself, we find no evidence for a tendency to lose the Schiff base proton, contrary to a recent suggestion[17]). We also note that some experimental evidence for a strongly polarised twisted singlet excited state does exist in retinol[18].

We suggest that the electrical signal revealed here occurs as the crucial, primary event in the photoprocess leading to vision. A crude classical estimate of the time required for the rotation of a rigid rotor, with moment of inertia equivalent to that of retinal, shows that the duration of such a signal is consistent with the 6 ps required for formation of prelumirhodopsin[19]. The electrical signal can act as a trigger on the medium, as the Z_1 state has 1–2 eV excitation energy which can be used as a source of energy to influence the system. There may be either a direct, electrostatic interaction on neighbouring 'pores', which could affect the disk membrane permeability to Na^+ ions, or an indirect interaction by way of induction of a conformational change in protein.

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Two new types of retinal degeneration in cerebellar mutant mice

HUMAN retinitis pigmentosa represents a class of diseases, the principal characteristic of which is the slow and progressive degeneration of photoreceptor cells. In several human neurological syndromes, photoreceptor degeneration accompanies cerebellar ataxia, deafness, mental retardation or other abnormalities. Some examples of such syndromes are Batten–Spielmeyer–Vogt disease, Refsum's disease, Laurence–Moon Biedl syndrome, Bassen–Kornzweig disease, Usher's syndrome and several of the mucopolysaccharidoses[1–4]. As far as we know laboratory animals with neurological syndromes that include photoreceptor degeneration have not been available.

We describe here two types of slow, progressive photoreceptor degeneration that have been discovered in two neurological mutant mice that lose cerebellar Purkinje cells. The first, Purkinje cell degeneration, gene symbol *pcd*, is a new autosomal recessive mutation that results in loss of virtually all Purkinje cells between the ages of 3 and 5 weeks[9]. The second is the nervous, *nr*, mutant which also loses Purkinje cells relatively early but to a lesser extent; up to 90% of the Purkinje cells in *nr*/*nr* mice degenerate between 3 and 7 weeks of age[5,6].

The *pcd* mutation occurred in the C57BR/cdJ strain but is now being transferred[9] to C57BL/6. The *nr* mutation occurred in the BALB/cGr strain and is coisogenic with that strain[5,6]. These two mutations are not linked, nor are they alleles of or linked to the retinal degeneration, *rd*, gene[9].

Homozygous mutants were identified by their ataxia resulting from the cerebellar defect at ages older than 15 and 22 d for *nr*/*nr* and *pcd*/*pcd*, respectively. Younger mutants and controls were identified by the cytological appearance of cerebellar Purkinje cells. Mice were reared in cyclic light[7]. Eyes of mutants and normal littermates at several ages were enucleated, fixed, embedded in plastic and sectioned as described elsewhere[7].

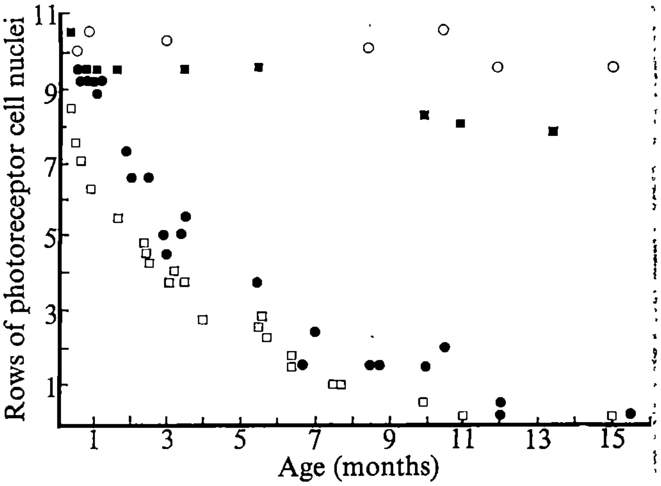

Fig. 1 Thickness of the outer nuclear layer measured as rows of photoreceptor nuclei at the posterior pole of the eye. Each point represents one animal: The number of rows of photoreceptor cell nuclei at the posterior pole of the eye (400–800 μm from the optic nerve head) was recorded as a range (for example, 4–5.5 rows), and the mid-point of the range was plotted. For both *pcd*/*pcd* and *nr*/*nr*, the average range was 1.5 rows. The cell loss in nervous controls may be an effect of albinism, light, heterozygosity for *nr* (oldest controls were known +/*nr*) or some interaction among these factors. □, *nr*/*nr*; ■, +/*nr* or +/+; ●, *pcd*/*pcd*; ○, +/*pcd* or +/+.

The time course of the retinal disease is similar in the two mutants although the loss of photoreceptor cells begins earlier and proceeds slightly faster in *nr*/*nr* than in *pcd*/*pcd* mutants (Fig. 1). Twenty-five to thirty per cent of the cells disappear by 3 weeks in *nr*/*nr*, but not until 2 months of age in *pcd*/*pcd*.

Retinae of *pcd*/*pcd* mice seem relatively normal at day 18, although there may be a slight increase over controls in the number of pyknotic photoreceptor cell nuclei. By day 25, however, there is an obvious increase in the number of pyknotic nuclei, and the rod outer segments are clearly disorganised (Fig. 2*a*; compare Fig. 2*h*). As the loss of photoreceptor cells

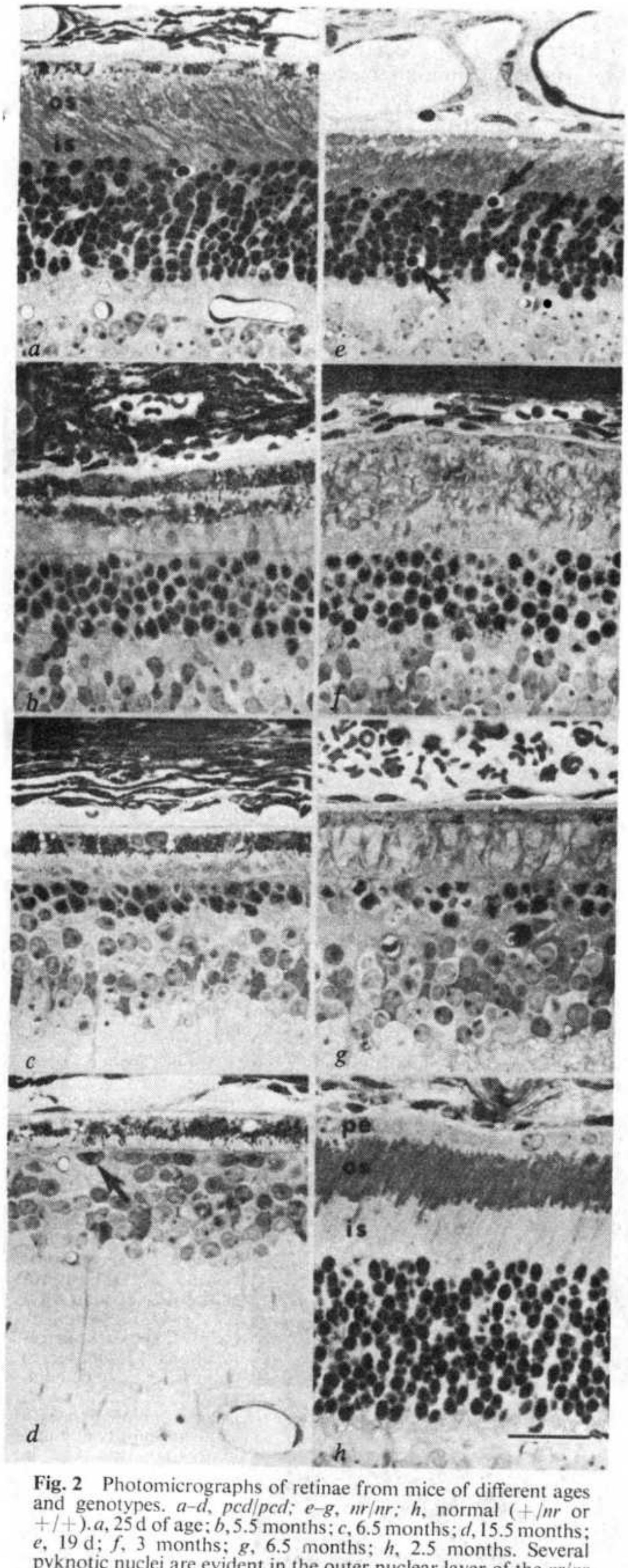

Fig. 2 Photomicrographs of retinae from mice of different ages and genotypes. *a–d*, *pcd*/*pcd*; *e–g*, *nr*/*nr*; *h*, normal (+/*nr* or +/+). *a*, 25 d of age; *b*, 5.5 months; *c*, 6.5 months; *d*, 15.5 months; *e*, 19 d; *f*, 3 months; *g*, 6.5 months; *h*, 2.5 months. Several pyknotic nuclei are evident in the outer nuclear layer of the *nr*/*nr* retina at 19 d of age (*e*, arrows), and the outer nuclear layer is thinner than that in normal retinae (*h*) or that in *pcd*/*pcd* mutant retinae of slightly older age (*a*). The outer segment layer is thicker in *nr*/*nr* retinae than in *pcd*/*pcd* retinae at stages of comparable photoreceptor cell loss (compare *f* with *b* and *g* with *c*). A residual photoreceptor cell is present after the rest of the outer nuclear layer has disappeared (*d*, arrow). The inner retinal layers appear relatively normal after photoreceptor degeneration, at least up to 16 months of age (*d*), is, inner segments; os, outer segments; pe, pigment epithelium. 1–2 μm Epon–Araldite sections. Toluidine blue. All magnifications the same; the bar represents 25 μm.

continues (Fig. 2*b–d*), the outer segments become shorter and more variable in length. There are regional differences in rate of photoreceptor degeneration in *pcd*/*pcd* similar to those observed in pigmented rats with inherited retinal dystrophy[7].

The retinal pathology of the nervous mutant is different. The youngest *nr*/*nr* animal we have examined was 13 d old, yet there are already many pyknotic nuclei, and the rod outer segments are disorganised and slightly shorter than those of littermate controls. These features are more conspicuous at day 19 (Fig. 2*e*). A few large whorls of outer segment membranes may be present as early as 3 weeks of age. These whorls accumulate so that by 2.5–3 months, the outer segment zone is comprised almost exclusively of whorls with few distinct outer segments (Fig. 2*f*). As a consequence of this accumulation, the outer segment zone in *nr*/*nr* mice is thicker than that in *pcd*/*pcd* mice with comparable cell loss (compare Fig. 2*b* and *f* with *c* and *g*) although the outer segments in *pcd*/*pcd* are usually more distinct. The outer segment zone in *nr*/*nr* becomes thinner after 3–4 months of age as additional photoreceptors are lost, and by 11 months no outer segment membranes are present. The membranous whorls in *nr*/*nr* resemble those seen in rats with inherited retinal dystrophy[7]. In a preliminary electron microscopic examination of *nr*/*nr* mice of several ages, mitochondria in photoreceptor inner segments were found to be larger in cross section than those of *pcd*/*pcd* or of controls; similarly, rounded mitochondria are an early feature in cerebellar Purkinje cells of *nr*/*nr* mice[6]. In *nr*/*nr* retinae the regional differences in the rate of photoreceptor degeneration are less striking than in *pcd*/*pcd*.

In both the *pcd*/*pcd* and *nr*/*nr* mice, photoreceptor degeneration consistently accompanied cerebellar ataxia. The 28 *pcd*/*pcd* and 29 *nr*/*nr* animals exhibiting ataxia had the appropriate type of retinal degeneration, and the 12 controls for *pcd*/*pcd* (+/*pcd* and +/−) and 34 controls for *nr*/*nr* (+/*nr* and +/−) had normal retinae. The retinae of other adult mutants with different cerebellar defects (reeler, weaver, staggerer and leaner) were examined; all were histologically normal.

The cerebellar disease in both *pcd*/*pcd* and *nr*/*nr* becomes evident about 2 weeks after birth and is virtually complete within a few weeks. Apart from this temporal similarity, however, the Purkinje cell lesions in the two mutants are cytologically distinct[6]. Retinal abnormality in both is first evident at about 2–3 weeks of age, but unlike the precipitous degeneration of cerebellar Purkinje cells, the visual cells disappear slowly during a year. It is obvious, however, that the retinal diseases of the two mutants are also distinct. The most notable differences are (1) the earlier onset in *nr*/*nr*; (2) the failure of *nr*/*nr* to develop rod outer segments at a normal rate; (3) the persistence of short, yet distinct rod outer segments in *pcd*/*pcd* compared with the accumulation of whorls of outer segment membranes in *nr*/*nr*; (4) the presence of enlarged mitochondria in *nr*/*nr* inner segments; and (5) the regional differences in rate of degeneration in *pcd*/*pcd* mutants. The significance of the differences in rate and distribution of the photoreceptor degeneration in *pcd*/*pcd* and *nr*/*nr* mutants is difficult to assess because the mutations are on different genetic backgrounds. The fact that *pcd* and *nr* are on pigmented and albino backgrounds respectively, is perhaps an important factor, since in rats with inherited retinal dystrophy the rate of degeneration is influenced by the amount of light to which the retina is exposed, either environmentally or as a result of differences in eye pigmentation[7].

Thus, *pcd*/*pcd* and *nr*/*nr* mutant mice exhibit two different types of retinal degeneration, similar in rate but probably different in aetiology. Unlike the rapid degeneration of photoreceptor cells in the *rd*/*rd* mutant mouse[8], these new retinal degenerations have a slow progression resembling that seen in the retinitis pigmentosa class of diseases in man. Analysis of the *pcd*/*pcd* and *nr*/*nr* mutants hopefully will provide insight into inherited retinal degenerations and their relationship to inherited degenerations of other neuronal classes in the central nervous system.

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Effect of denervation and local damage on extrajunctional L-glutamate receptors in locust muscle

EXTRAJUNCTIONAL receptors occur in both normal vertebrate muscle[1] and locust muscle[2] as demonstrated by iontophoretically applied acetylcholine (ACh) and L-glutamate respectively. Two types of extrajunctional glutamate receptors occur in locust muscle in addition to the junctional receptors[3]. The biphasic response (depolarisation preceding hyperpolarisation) seen when glutamate is applied iontophoretically to extrajunctional regions of locust muscle results from simultaneous activation of the two types of extrajunctional glutamate receptors[3]. The D receptor (which mediates a depolarisation) is activated by L-glutamate but insensitive to the glutamate analogue DL-ibotenate, while the H receptor (which mediates a hyperpolarisation) is activated by both L-glutamate and DL-ibotenate. This pharmacological difference has allowed separate investigation of the two types of receptors.

Following denervation vertebrate muscle develops a high degree of extrajunctional sensitivity to ACh[4,5], whereas locust muscle develops a high degree of extrajunctional sensitivity to glutamate[6]. The transection of vertebrate muscle causes the development of extrajunctional sensitivity to ACh at the site of damage[7]. It was therefore of interest to see whether both types of extrajunctional receptors normally present in locust muscle are involved in the changes in sensitivity of the extrajunctional membrane after denervation, and to determine if similar changes occur after muscle damage.

Extrajunctional glutamate receptors were investigated in the extensor tibiae muscle of adult locusts (*Schistocerca gregaria*). Receptors in normal muscles were compared with those in muscles which were either denervated by transection of the nerve supply[6] or were damaged locally with a single scalpel cut. The sensitivities of extrajunctional receptors were mapped using double-barrel micropipettes of high resistance[3,8,9] (150–300 MΩ) which contained L-glutamate (sodium salt, 0.1 M, *p*H 8) in one barrel and the glutamate analogue DL-ibotenate[10] (sodium salt, 0.1 M, *p*H 7) in the other. Micropipettes were moved in 10 or 20 μm steps across muscle fibres (~ 100 μm wide), or along sections of the outer faces of the fibres. Glutamate, which activates both types of receptors, was used to measure D-receptor sensitivity in situations where the D response was the predominant one. H-receptor sensitivity was obtained with ibotenate, which activates only H receptors. In situations where the sensitivity of the D receptors was low, the H receptors were first desensitised with ibotenate so that glutamate now activated only the D receptors to give an accurate value for their sensitivity. As the H response had an equilibrium potential which lay close to the resting potential of the muscle fibres, care was taken that

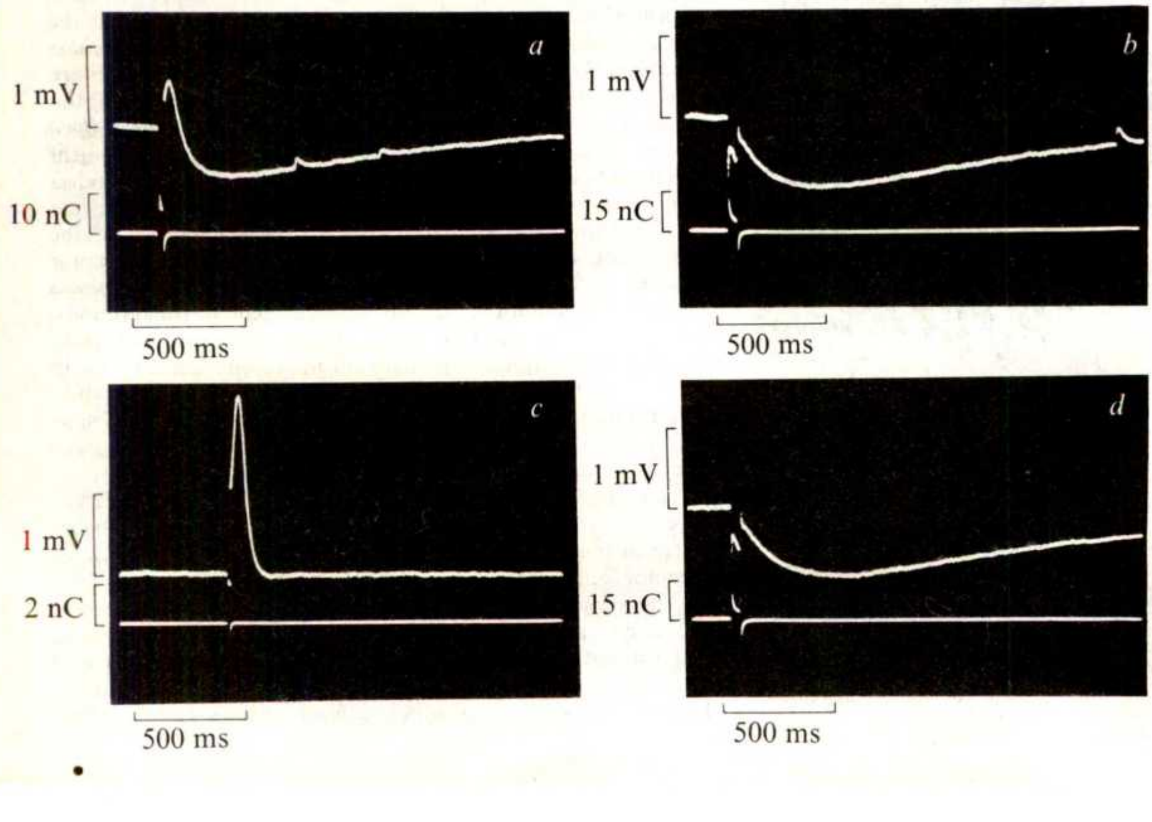

Fig. 1 The effect of denervation on the extrajunctional response to iontophoretically applied glutamate and ibotenate, applied from adjacent barrels of a high resistance micropipette. Drug ejection currents passing through the micropipette are monitored on the lower traces. *a*, Control muscle, extrajunctional response to glutamate; a two component response results from the activation of both types of extrajunctional receptors; *b*, control muscle, extrajunctional response to ibotenate; a single component H response results from the activation of the H receptors; *c*, denervated muscle, extrajunctional response to glutamate; a single component D response is present. Note that a smaller quantity of glutamate than was used in *a* produces a greatly increased D response. The quantity of glutamate used is insufficient to produce a significant H component; *d*, denervated muscle, extrajunctional response to ibotenate; a single component H response unchanged from the control muscle is present. Miniature excitatory potentials are seen on the traces before denervation.

this did not introduce error in sensitivity measurements.

Both denervation and local damage of the muscle fibres resulted in increased extrajunctional sensitivity to glutamate in animals kept at room temperature for 10 to 14 d. Following denervation, the entire extrajunctional membrane developed a uniform increased sensitivity to glutamate. Thus the iontophoretic application of glutamate, to denervated muscle fibres, in quantities smaller than those normally needed to produce an extrajunctional response in innervated muscle, resulted in a depolarisation with a short latency (Fig. 1). If the quantity of glutamate applied to the denervated muscle was as large as that normally used to produce an extrajunctional response in innervated muscle, a biphasic response was obtained, although the H component was partly masked by the large D component. The iontophoretic application of ibotenate demonstrated the presence of the H component in denervated muscle fibres. As the increased sensitivity initially developed in a non-uniform manner, it was possible to compare the relative sensitivities of the two types of extrajunctional receptors in regions of the same muscle fibre which differed in sensitivity. In such situations, although the D-receptor sensitivity varied severalfold in different regions, the H receptors demonstrated no significant change and gave values which fell within the ranges encountered in normal control muscles (see Fig. 2).

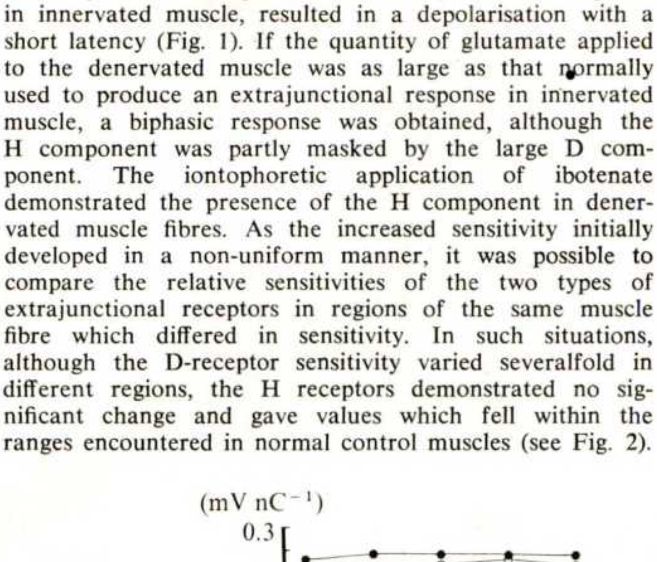

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Fig. 2 Graph of the distribution of extrajunctional receptor sensitivity in a muscle fibre after denervation. Inset shows the sensitivity of extrajunctional glutamate receptors in a typical innervated fibre from a control muscle (80 μm wide). ●, D-receptor sensitivity to glutamate. ○, H-receptor sensitivity to ibotenate. Abscissa, distance across the muscle fibre surface.

Locally damaged fibres[7] developed an increased extrajunctional sensitivity to glutamate which was highest at the site of local damage and fell to normal levels within ~ 100 μm. The H receptors demonstrated no measurable change in sensitivity in different regions of the same fibre, giving values similar to control muscles, whereas the D receptors changed by approximately 100-fold from 5 mV nC^{-1} at the site of injury to 50 μV nC^{-1} in other parts of the same fibre.

The increased sensitivity involved receptors which mediated ionic permeability changes to Na^{+}/K^{+}. In this respect they resemble the extrajunctional D receptors in innervated muscle[2]. The H responses produced by ibotenate, applied iontophoretically to extrajunctional regions of high glutamate sensitivity, had equilibrium potentials similar to those obtained in normal control muscles indicating increased Cl^{-} permeability[3].

Thus, when extrajunctional sensitivity to glutamate was altered by two different methods, that is, denervation and mechanical damage, the high degree of sensitivity which developed was the result of an increased sensitivity of the D receptors with no apparent change in H receptors. This, and the observation that, following denervation and muscle damage, D receptors varied considerably in sensitivity in different parts of the same fibre while the sensitivity of the H receptors remained unaltered, demonstrates that the two types of extrajunctional receptors can change independently.

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Relationship of spontaneous fibrillation potentials to muscle fibre segmentation in human muscular dystrophy

DENERVATED skeletal muscle fibres have long been known to show spontaneous fibrillation potentials, this being one of the features resulting from the removal of the trophic influence of the motor nerve[1]. Electromyography (EMG) has shown spontaneous fibrillations in human myopathies such as myositis or muscular dystrophy[2–6], but their true incidence and mechanism is still obscure. We propose that myopathic fibrillations result from segmental necrosis of muscle fibres so that a distal fibre segment is separated from the part carrying the motor endplate. We show here, first, that after experimental myotomy in the baboon biceps muscle, the nerve-free segments develop fibrillation after a consistent delay and second, that by comparing different clinical types of human myopathies a correlation can be found between the incidence of focal necrosis and spontaneous fibrillation potentials. Our findings are consistent with our previous report of collateral innervation of newly formed nuscle fibres in Duchenne muscular dystrophy[7].

Experimental myotomies were performed in sterile conditions in seven healthy adult baboons or macaques, under light pentothal anaesthesia. EMG tests and biopsies were then carried out and the animals were not killed. The biceps brachii muscle was used because its motor endplates are concentrated in a narrow innervation zone[8] which can be identified on the exposed muscle by focal stimulation with a sterile steel electrode placed on the superficial muscle fibres. Threshold current pulses of 0.1 ms duration (about 1 mA) elicited brisk twitches at the motor point but the same stimuli were ineffective when sited more distally on the same bundles (Fig. 1*a*). A number of small cuts were made in several muscle bundles with iridectomy scissors, at least 10–15 mm distal to the motor point thus defined. We

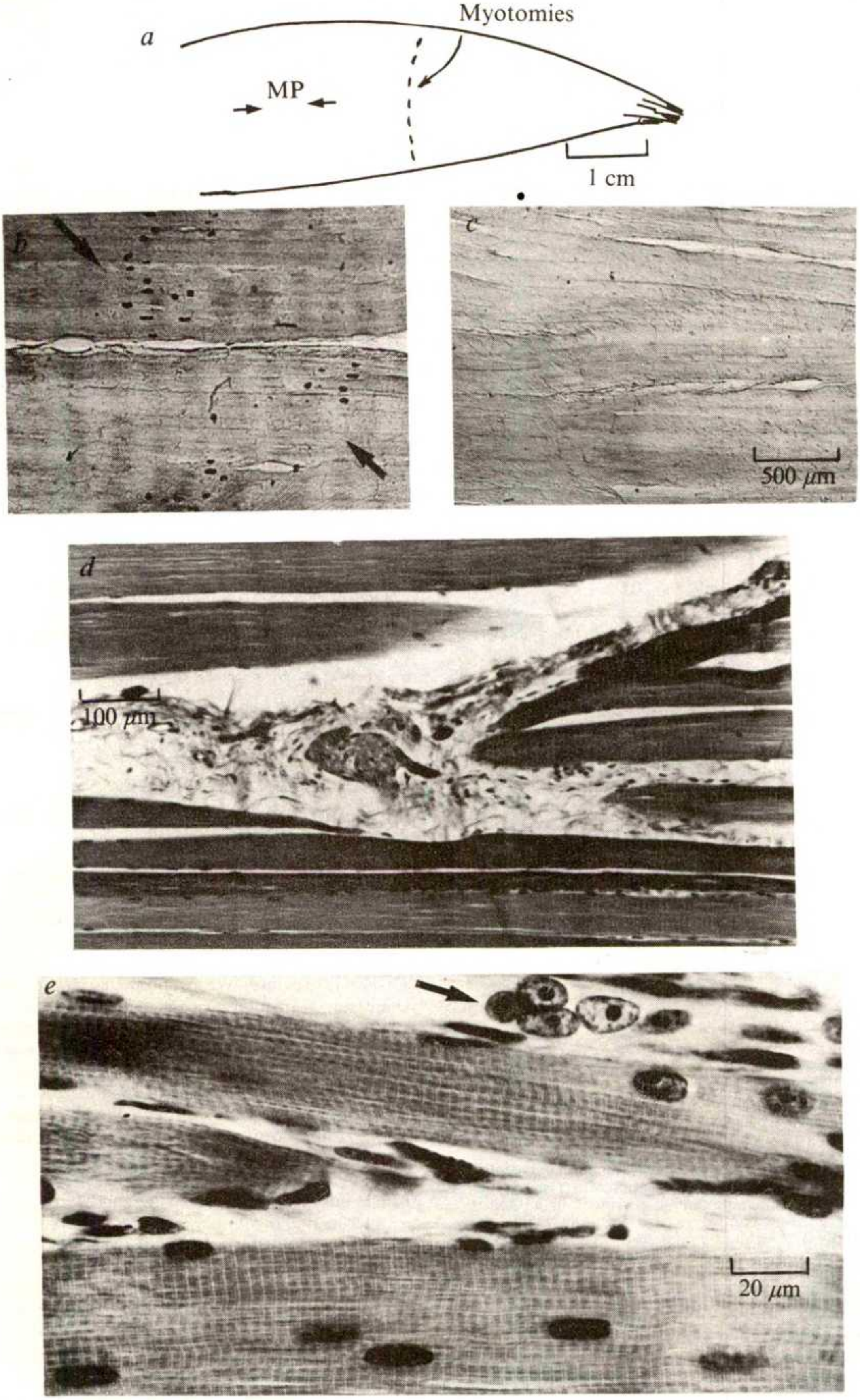

Fig. 1 Longitudinal histological sections in baboon biceps biopsied 2 months after extrajunctional myotomies. *a*, Sketch of the muscle with the motor point (MP) located by electrical stimulation and the distal myotomies. *b* and *c*, Acetylthiocholine method for staining motor endplate cholinesterase. *b*, Motor point region with many endplates (between arrows). *c*, Distal biceps near myotomies with no cholinesterase accumulations. *d* and *e*, Sections through myotomy area stained with Masson trichrome. *d*, Segmented muscle fibres (middle) next to intact muscle fibres (below). *e*, Myotube with four myoblasts (arrow).

were careful not to cause any lesion to the blood supply. The aponeurosis and skin were sutured. One to two months after the physiological observations a muscle biopsy was removed and longitudinal sections 5–10 μm were made. Staining for cholinesterase by the acetylthiocholine method disclosed motor endplates at the level of the previously identified motor point (Fig. 1*b*) whereas no focal cholinesterase accumulation was found throughout the distal part of the biopsy (Fig. 1*c*). Obviously the myotomies failed to involve any motor nerve fibre because they were quite distal to the innervation zone and intramuscular nerves. Some biopsy specimens were also stained with Masson trichrome, showing fairly normal isolated muscle fibre segments at the level of the myotomy (Fig. 1*d*). The segments had restored their membrane at the level of the lesion where myoblast proliferation and myotube formation were seen, with typical vesicular nuclei containing prominent nucleoli (Fig. 1*e*).

After the operation the animals were tested repeatedly after receiving a small intramuscular dose of tranquiliser (Sernylan or Thalamonal). The biceps muscles were explored with a sterile concentric needle electrode inserted through the skin, as for a human EMG. No fibrillation potentials were observed in the first 5 d. After 5 to 6 d, abundant fibrillations of typical triphasic waveform were recorded in many sites of the operated biceps, but only distally with respect to the myotomies (Fig. 2). No fibrillation was detected above that level where typical motor unit potentials could be elicited by stretching the muscle slightly. The presence of the needle electrode in the muscle did not trigger the fibrillation potentials because the latter could readily be demonstrated with subcutaneous steel needles which did not enter the muscle itself (Fig. 2*a*). A few of the muscles were not tested before the tenth day to ensure that the fibrillations then recorded could not be ascribed to an inadvertent lesion possibly related to electrode insertions. The distal muscle fibre segments which fibrillated had obviously been deprived of neural influence since the time of the mytomy. On the other hand, when muscle fibres are denervated by neurotomy, the distal nerve stump takes about 2–3 d to degenerate in the baboon[10] and it would be expected to maintain some trophic action on the muscle during this time[11]. Two animals were prepared with biceps myotomy on one side and with a partial section of the biceps nerve on the other side. Spontaneous fibrillations started in the distal biceps with myotomy about 2 d earlier than in the partially denervated biceps. About 100 different fibrillation potentials were recorded after 10–20 d on either side in these animals. Most potentials were triphasic and their mean total duration was 3.1 ± 1.0 ms. The mean voltages were 136 ± 76 μV on the myotomy side and $112+53$ μV on the partially denervated side ($P < 0.01$). Whereas in devervated muscle fibres, fibrillations generally arise at the site of the old endplate[12] this is not an absolute rule[1] and our results indicate that fibrillations are generated in nerve-free segments of primate muscle (Fig. 2). Other changes characteristic of removal of neural trophic factors had been found in frog muscle segments—the appearance of acetylcholine receptors[13] and the acceptance of motor innervation[14].

The hypothesis that myopathic fibrillations result from muscle fibre segmentation is further supported by the finding

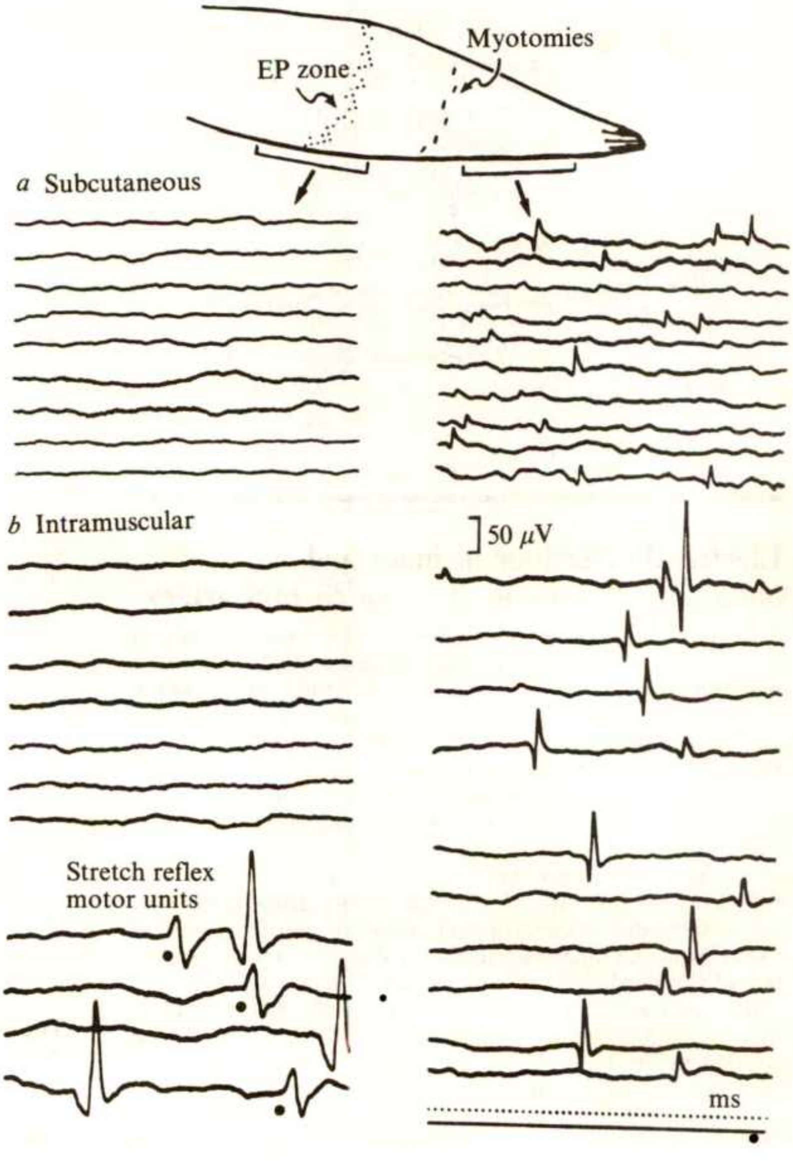

Fig. 2 Cathode ray oscillograms from baboon biceps muscle, 10 d after extrajunctional myotomy. Recording from the motor endplate zone (left column) and from the region distal to myotomy (right column). *a*, Subcutaneous bipolar needles not penetrating into the muscle. *b*, Concentric needle electrode in the muscle. Same calibration for all records. Fibrillation potentials are only recorded in distal region. At the bottom of the left column, a slight stretch elicited motor unit potentials and the one marked by dots starts with a negative (upwards) component which indicates focal recording at the endplate (see refs 8 and 9).

that spontaneous fibrillations are much more abundant than hitherto suspected in Duchenne muscular dystrophy[4,15], whereas they were very scarce, if present at all, in the muscles of five patients with typical facio-scapulo-humeral (Landouzy–Dejérine) muscular dystrophy. The most important pathological change in Duchenne muscular dystrophy is focal necrosis which isolates long fibre segments from the endplate region[16,17] whereas this lesion is rare in facio-scapulo-humeral muscular dystrophy[17]. The correlation also holds for the muscles of patients with acute polymyositis which have marked lesions of focal necrosis[18] as well as abundant fibrillations in EMG tests. Because they generally looked at transverse muscle sections most pathologists failed to emphasise that myopathic necrotic lesions, far from destroying the muscle fibres, result in serially arranged fibre segments. That nerve-free segments thus formed are collaterally innervated and can then be activated by the motor nerve in Duchenne muscular dystrophy[7,15], and also in polymyositis[19], sheds new light on the pathogenesis and emphasises regenerative capabilities which must retard the clinical progression and/or promote the recovery of human myopathies with muscle fibre segmentation.

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Electrical behaviour of inner and outer smooth muscle of sheep carotid artery

MICROELECTRODE studies of inner smooth muscle cells of unstimulated rabbit carotid arteries show a stable membrane potential[1,2], as do sucrose-gap records from sheep carotid arteries[3,4], the latter method records only from cells that are widely interconnected electrically. Information about the electrical behaviour of the outer muscle of arteries is particularly important as only the outer muscle is innervated, and it is much less sensitive than inner muscle to vasoconstrictor agents[5].

We have now been able to record from the outer, as well as the inner, muscle of the sheep carotid artery by microelectrodes after stripping away successive layers of tissue from the inner side of the artery. Electrotonic potential was applied through external electrodes[6]. The strips of artery used were approximately 3 mm wide and 15 mm long, their long axis being aligned round the artery wall and parallel to the long axis of their smooth muscle cells. The bathing solution contained (mM): Na^+ 137.4; K^+ 5.9; Mg^{2+} 1.2; Ca^{2+} 2.5; Cl^- 134.0; HCO_3^- 15.5; $H_2PO_4^-$ 1.2 and glucose 11.5. It was gassed by 95% O_2 : 5% CO_2 and maintained at 36–37 °C.

Microelectrodes inserted into inner cells through the intact intimal surface of the vessel recorded a stable membrane potential of -59.5 ± 5.3 mV (mean ± s.d., 33 experiments). Spontaneous electrical activity was never observed in these cells. Electrotonic hyperpolarising currents caused hyperpolarisation proportionate to the current intensity.

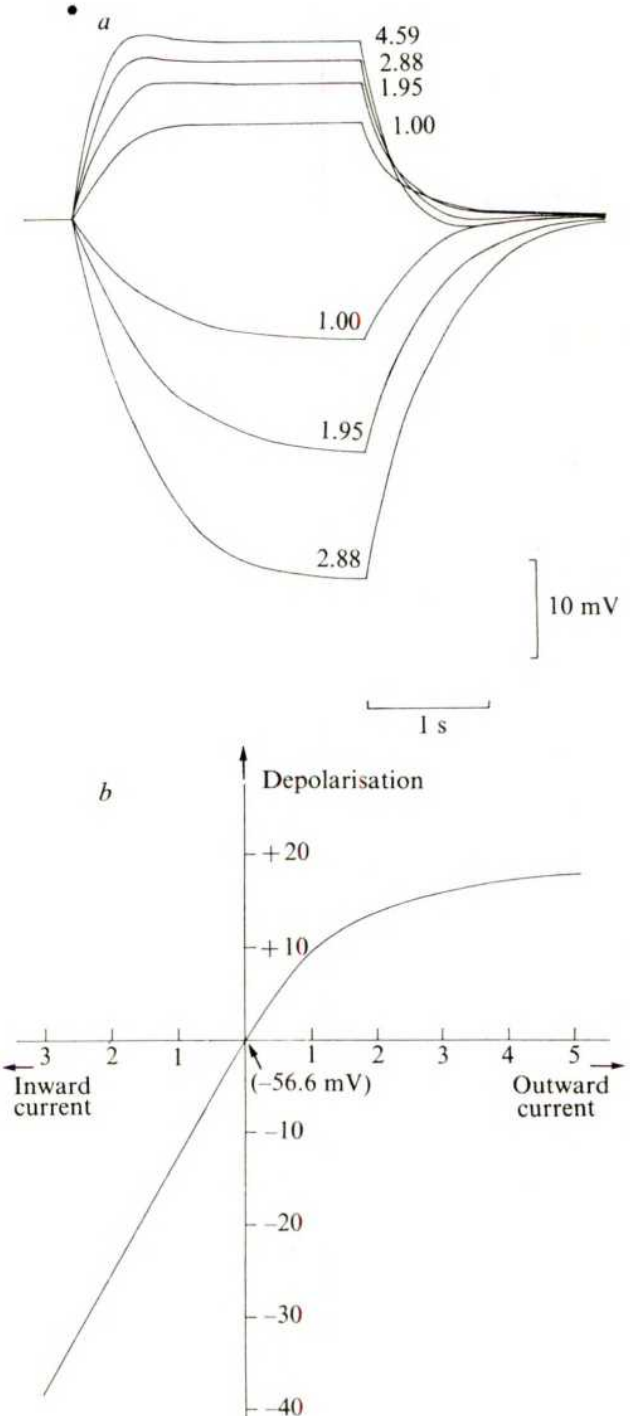


Fig. 1 *a*, Electrotonic potential evoked by external current application to the inner muscle of the sheep carotid artery. *b*, Voltage–current relationship for steady state readings. Values for current in arbitrary units.

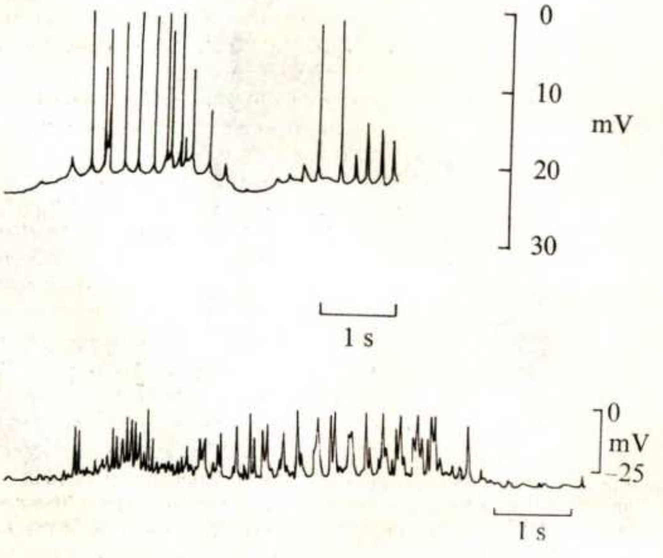
Depolarising currents caused proportionately less depolarisation when the membrane potential passed approximately −40 mV and the final level of depolarisation was then often less than was initially produced (Fig. 1). This delayed rectification on depolarisation is very similar to that seen in inner cells of rabbit aorta[7] in which it was attributed to an increase in potassium permeability. Depolarising currents never produced a series of electrical discharges. Similar results were obtained from inner muscle cells after stripping off the intima and a layer of adjacent muscle about 50 μm thick; membrane potential was stable at 60.0 ± 5.5 mV (mean ± s.d., 30 experiments) and electrotonic current produced changes similar to those in Fig. 1. The space constant of inner muscle measured by electrodes inserted at increasing distances from the polarising electrodes was always between 1.74 and 2.43 mm (Table 1) which is similar to the value of 1.26–3.49 mm obtained for these arteries by the sucrose-gap method[8].

Table 1 Membrane constants of smooth muscle of the sheep carotid artery

Intact inner muscle		Stripped inner muscle	
Experiment no.	Space constant (mm)	Experiment no.	Space constant (mm)
1	1.75	2	2.04
3	1.77	6	1.98
4	1.87	8	2.14
5	2.00	13	2.18
7	1.99		
9	1.73		
10	2.27		
11	1.74		
12	2.43		
Means	1.95 (s.d. ± 0.24)		2.09 (s.d. ± 0.08)

When further layers were stripped away from the inner part of the artery wall and microelectrodes inserted from the stripped surface into the outermost muscle, irregular spike discharges were recorded in every case (Fig. 2), which were sometimes superimposed on slow oscillations. The membrane potential between spikes was only −31.1 ± 4.4 mV and spike height was 28.8 ± 5.7 mV (means ± s.d., 30 experiments). Overshoot of the spike beyond zero potential was rare. No electrotonic potential could be induced in the outer cells even with the nearest polarising electrode only 0.25 mm from the recording site. Attempts to record from outer muscle by inserting microelectrodes from the outer surface, after stripping the adventitia, were not successful.

Fig. 2 Action potentials recorded from the outer muscle of sheep carotid artery.



The low resting potential and spontaneous activity of outer muscle might have resulted in part from the stripping of adjacent tissue that was necessary to expose it; however, as a similar procedure failed to induce any activity in inner muscle, the outermost cells of the artery clearly developed electrical activity more readily than inner cells. They also differed in lacking widespread electrical contacts with neighbouring cells. These properties would allow electrical discharges in outer muscle to have an important, and localised, function in mediating its response to nerve. The relative electrical stability of inner muscle, attributable to powerful delayed rectification, and long space constant, make it adapted for extensive conduction of steady depolarisation such as that induced by injury[8].

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Possible biochemical explanation for specific reformation of synapses between muscle and regenerating motoneurones in cockroach

THE reformation of synaptic connections by a neurone whose axon has been severed often proceeds with great specificity so that synapses are reformed only with the original cell. It is possible that this occurs as a result of a specific recognition process at the surface of the cells. Evidence for such a process can be obtained by biochemical analysis. The neuromuscular system of the cockroach *Periplaneta americana* leg is a system which demonstrates specificity of synapse formation by regenerating motoneurones[1] and which lends itself to such an investigation.

The set of coxal depressor muscles (CDMs) in each leg are composed of individual muscles each of which can be identified and numbered[2]. Six of the CDMs in the metathoracic legs, 178, 179, 177d′, 177d, 177e′ and 177e are particularly suitable for such studies because their pattern of innervation by identified motoneurones in the metathoracic ganglion has been determined[3]. All the fibres in muscles 178 and 179 are innervated by the same identified, fast motoneurone, cell 28 in the map by Cohen and Jacklet[4]. In muscles 177d and 177e all the fibres are innervated by the same slow motoneurone on the dorsal surface of the ganglion, probably cell 52, and in addition by three inhibitory motoneurones. Muscles 177d′ and 177e′ possess the unusual characteristic of having every fibre innervated by both motoneurone 28 and 52 (ref. 3) so that they are an homogeneous population of doubly innervated fibres with no inhibitory innervation. Another advantage to using this cockroach system is that it has been demonstrated that after the nerves to the CDMs have been cut and regeneration of the neurones has proceeded so that movement is recovered, the excitatory motoneurones have specifically reinnervated the original muscles[5]. This is the only system where specificity of synapse formation during regeneration has been demonstrated at the level of individual, identified cells. The CDMs are large enough for some biochemical analyses to be done on tissue from a single cockroach. The amount of protein in the various CDMs from the adult male cockroach is sufficient for polyacrylamide gel electrophoresis of muscle proteins from a single animal but not sufficient for biochemical studies requiring bulk amounts of material (178, 179, 1.3 mg; 177d, 80 μg; 177d′, 70 μg; 177e, 60 μg; 177e′, 90 μg).

If the regenerating motoneurones make synapses with the correct muscle as a result of a specific recognition involving macromolecules on the surface of the muscle then one should be able to distinguish biochemically the various CDMs. This was surprisingly easy to do. When unfractionated homogenates of each of the six CDMs are analysed by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis[6], three different gel protein patterns are observed (Fig. 1). All the muscles possessing one particular protein pattern also have the same innervation. In the doubly innervated muscles, 177d′ and 177e′, the gel protein pattern seems to be a composite of those protein bands correlated with each of the two individual motoneurones. Innervation of a muscle by motoneurone 28 is accompanied by the bands labelled F1, F2, whereas innervation by motoneurone 52 is accompanied by bands S1, S2, S3, S4, S5, S6, S7. Innervation by the inhibitory motoneurones is accompanied by band S8. More than fifty individual cockroaches have been examined in the course of this study and the association of a particular gel protein pattern and innervation type has been observed in every one.

If any of these protein differences are contributing to the specific recognition of the CDMs by the regenerating motoneurones it must be demonstrated that they are present in the muscle throughout the period of neural regeneration and that their synthesis by the muscle is not controlled by the motoneurone. The nerves were therefore cut and the denervated CDMs analysed at various times after the operation by SDS–polyacrylamide gel electrophoresis as in Fig. 1. No change is observed in the gel protein pattern of the six CDMs as a result of denervation up to 8 weeks after the operation. The nerves were recut every 3 weeks to make sure that the motoneurones did not reconnect with the muscles. Eight weeks after denervation, the muscles have atrophied to such an extent (less than 40% normal protein content) and become so covered with fat cells and haematocytes that a clean dissection of muscle tissue is impossible. This experiment therefore demonstrates that throughout the period of regeneration of motoneurones (30–60 d for initial contact) the six CDMs can always be distinguished by their gel protein patterns.

The failure of denervation to change the gel protein pattern of the CDMs may be because the proteins have a long half life or are continually synthesised. The nature of newly synthesised proteins from the CDMs was examined by measuring the distribution of radioactivity in the SDS electrophoresis gel after running a sample containing ^{3}H-leucine incorporated into muscle proteins. It is observed (Fig. 2a) that the pattern of radioactivity in the innervated, control CDMs is not identical with that pattern of stained bands. Discrepancies may be accounted for by different turnover rates for the various proteins. Although it was very easy to distinguish the different CDMs by their gel protein pattern, it is more difficult, but still possible, to do so by examining the patterns of radioactivity. When the radioactive proteins of molecular weight less than 35,000 are examined on an expanded scale however, the differences become more apparent (Fig. 2*b*). The different amounts of F1, S1, S2, S3 synthesised by the various CDMs are clearly seen. Although the patterns of newly synthesised proteins are changed when the CDMs are denervated (Fig. 2*c*), they can still be clearly distinguished from each other as seen in Fig. 2*d*.

Mammalian skeletal muscle responds quite differently from cockroach muscle to denervation. On denervation of mammalian slow or fast skeletal muscles physiological and biochemical parameters change to values intermediate between those of normal fast and slow fibres[7]. An apparent dedifferentiation of both types of fibres to a common, simplified type has occurred. This loss of relative biochemical identity may account for the lack of specificity seen in its interactions with regenerating mammalian motoneurones which seem to be able to reinnervate any denervated muscle[8]. It is from these differences in response to denervation that we seek to explain the specificity observed in the cockroach neuromuscular system. The observed ability of the various CDMs to maintain their biochemical identity after denervation and throughout the entire period of regeneration of motoneurones must be an absolute requisite for specific reinnervation to occur by a process requiring a specific recognition between the cells.

Although the various CDMs have different mechanical

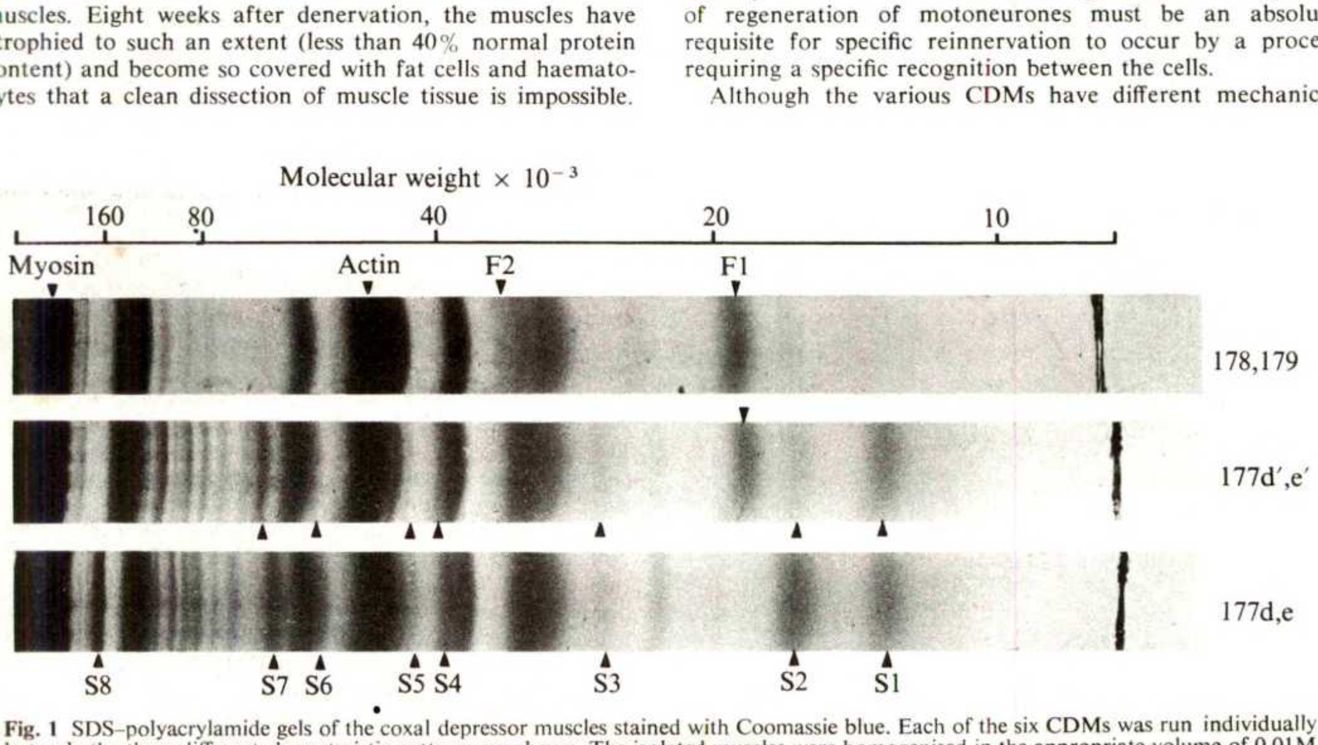

Fig. 1 SDS–polyacrylamide gels of the coxal depressor muscles stained with Coomassie blue. Each of the six CDMs was run individually but only the three different characteristic patterns are shown. The isolated muscles were homogenised in the appropriate volume of 0.01 M Tris-HCl (*p*H 7.4), 10% sucrose, 1% SDS, and 5% 2-mercaptoethanol and heated in a boiling water bath for 5 min. Aliquots of these solutions were applied to 10% polyacrylamide gels which were run in the presence of 0.2% SDS, stained with Coomassie blue, and destained all according to the methods of Fairbanks *et al.*[6].

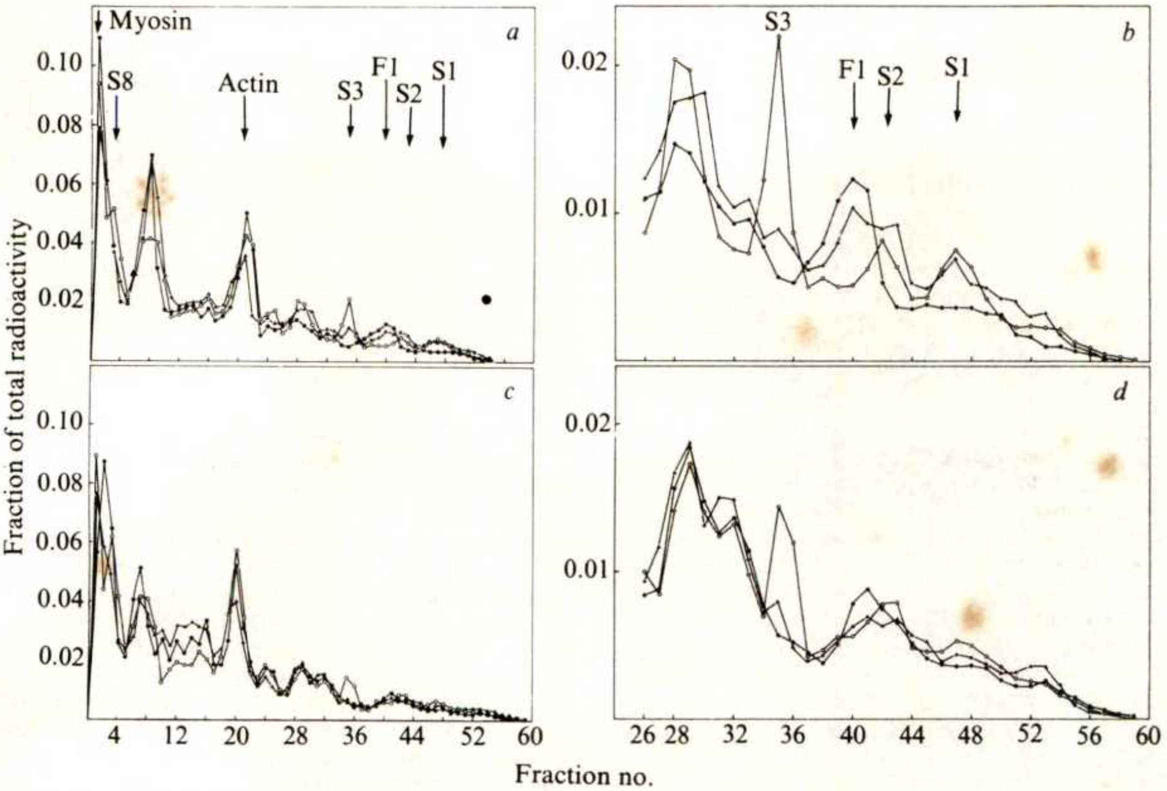

Fig. 2 Distribution of radioactivity after SDS–polyacrylamide gel electrophoresis of CDM homogenates after ^{3}H-leucine incorporation *in vivo*. Innervated control CDM (*a*) and (*b*) and CDM denervated 4 weeks before experiment (*c*) and (*d*) are taken from different metathoracic legs of the same animal. (*b*) and (*d*) are portions of (*a*) and (*c*) respectively but with expanded axes. The various CDMs are 178, 179 (●); 177d, 177e (○); 177d′, 177e′ (△). ^{3}H-leucine (specific activity 46 Ci mmol^{-1}) was concentrated twentyfold and 5 µl containing 117 µCi were injected into the coxa of each metathoracic leg just above the CDMs. After 22 h the muscles were removed, homogenised in sample solution, prepared for electrophoresis, run on gels, run through staining procedure (without Coomassie blue), and destained as described in Fig. 1. The gels were sliced into 1-mm sections and allowed to dry overnight. One millilitre of NCS (10% water) tissue solubiliser was added to each slice in a vial and they were incubated at 45 °C for 48 h. Toluene scintillation fluid was added and samples counted. Double-labelling experiments were not done because it was felt to be more important to have control, innervated and experimental, denervated muscles come from the same animal. The techniques were sufficiently precise that qualitatively similar results were obtained in each of three experiments.

properties it has been demonstrated that these are produced by differences in neuromuscular transmission and not by different contractile mechanisms in the muscle[9]. Therefore, the protein differences observed are not likely to be involved with contractile processes. It has not been demonstrated that any of the protein differences have a functional role in the specific recognition of the muscle by the regenerating motoneurone. It seems highly likely, however, that if such proteins exist they would be expected to express the following properties. (1) Their presence in a muscle should be associated with innervation by a particular motoneurone. (2) They should be present in the muscle throughout the period of regeneration of the motoneurones. (3) Their synthesis should be an endogeneous property of the muscle and not under trophic control of the motoneurone. The protein bands described in this communication that serve to characterise a particular CDM possess these properties.

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Skin collagen allografts in the rat

THE factors involved in skin graft rejection have not been elucidated fully but it is known that the cellular components of skin, including the epidermis and its appendages, vascular endothelium and passenger leukocytes, are of primary importance in the process[1]. Such cellular elements can be removed by treating skin with a solution of crystalline trypsin to leave the dermal collagen component intact. A preliminary study of allografts of trypsin purified dermal collagen (hereafter referred to as dermal collagen) in the pig showed that although subcutaneous implants underwent some initial collagenolysis they achieved a state of permanence without lymphocytic infiltration occurring[2]. Since this concurred with the view that mature collagen shows low or no antigenicity, especially within species[4–7], we thought it likely that trypsin prepared collagen could be transplanted in animals other than the pig without eliciting an immune response.

Here we report on the fate of dermal collagen allografts, using the same outbred strains or differing strains of rat as donors and recipients, in an evaluation of dermal collagen

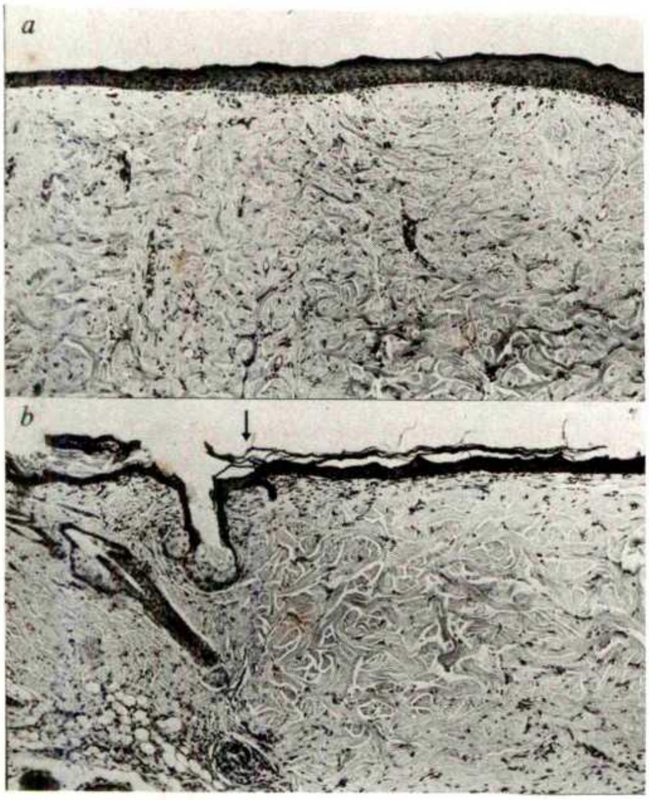

Fig. 1 Trypsin prepared ASH/W dermal collagen, treated with 0.01% glutaraldehyde then grafted into full thickness loss excised skin wounds in Porton recipients (haematoxylin and eosin, ×135). *a*, 28 d after grafting. The recipient epidermis which has migrated over the graft is hyperplastic and has a parakeratotic surface layer. *b*, 27 d after grafting. Recipient skin is at left of graft junction (arrow). The epidermis over the graft is of nearly normal thickness and has a stratum corneum. Dermal collagen was prepared by treating full thickness skin with a solution of crystalline trypsin (Koch-Light) at a concentration of 2 mg ml^{-1} in 0.1 M phosphate buffer solution, *p*H 9.0, with 0.5 mg ml^{-1} sodium azide as a bactericide at 15 °C for 28 d. The collagen was then treated with 0.01% glutaraldehyde (Taab Laboratories) in Tris buffer, *p*H 7.2 for 16 h at 15 °C before washing and grafting. Glutaraldehyde is known to desensitise collagen to the activity of collagenase[2].

for the replacement of collagenous tissues and, in particular, skin.

To confirm skin antigenicity, 31 skin allografts and 15 autograft controls were grafted into subcutaneous sites or excised skin wounds, biopsied 5–84 d after operation and examined histologically. In contrast with the autografts, the allografts developed the characteristic features of graft rejection, that is, rapid infiltration with lymphocytes, destruction of the epidermis and its appendages within 7–14 d and variable lysis of the graft collagen.

A total of 84 dermal collagen allografts were then implanted subcutaneously and biopsied 3–83 d after operation. These included 44 implants pretreated with solutions of glutaraldehyde at concentrations ranging from 0.001 to 1.0%. The implants, which were all recovered intact, became recellularised and revascularised with no overt evidence of lymphocytic infiltration or of collagenolysis. The original collagen bundle architecture was especially well preserved in implants treated with 0.01% glutaraldehyde, but persistent inflammation was associated with those treated with higher concentrations. The antigenic status of dermal collagen was investigated further by sensitising six rats to allogenic skin by making subcutaneous implants 14 or 28 d before implantation of collagen from the same donor. The collagen implants, which were recovered 14–48 d after operation, were no different from those implanted in non-sensitised rats and in particular showed no signs of lymphocytic infiltration.

Having established that allograft dermal collagen was neither resorbed nor induced an immune response in subcutaneous sites, we then investigated the possibility of incorporating dermal collagen into cutaneous wounds. Thirty-two collagen allografts (including 10 pretreated with 0.01% glutaraldehyde) were grafted into full thickness loss excised wounds which were examined, measured and biopsied 4–59 d after operation.

It was important to minimise graft desiccation and a foam dressing as used on five of the grafts proved ineffective in this respect. Ten of the wounds (seven measuring 5×5 mm and three measuring 10×10 mm) were simply covered with an overlapping sheet of dermal collagen then dressed with Vaseline petroleum jelly gauze. Here the outer aspect of the collagen became desiccated but the underlying collagen not only became recellularised and revascularised but also progressively epidermalised with recipient epidermis (Fig. 1*a*).

A further 17 10×10 mm wounds received fitting collagen grafts and were dressed with a sheet of dermal collagen then Vaseline gauze. The collagen dressings desiccated and since the grafts became epidermalised by migration either at the graft–dressing boundary or within the superficial aspect of the grafts the whole or greater thickness of the grafts became incorporated into the wounds (Fig. 1*b*). Epidermalisation resembled that described for healing incisional wounds[8,9] except that it occurred over a large area and took 4–5 weeks for completion in the 10×10 mm grafts. The wound epidermis was initially hyperplastic with an outer parakeratotic layer (Fig. 1*a*) but, eventually assumed a normal thickness with a stratum corneum (Fig. 1*b*).

The grafts as measured 3–8.5 weeks after operation, ranged from 60 to 100% of their original area, with best size maintenance in the 0.01% glutaraldehyde treated grafts. The efficacy of the grafts in suppressing wound contraction can be demonstrated by comparing them with excised wounds and full thickness autografts of the same initial size. Excised wounds without dressings contracted to hairline scars by 14 d and by 32 d when covered with a Vaseline gauze dressing. Autografts maintained their size for 2–3 weeks then diminished to 50–60% of their original area by 5 weeks (figures of 45–55% have been reported in a comparable study[10]).

The desiccated collagen dressings (or superficial aspect of the grafts) formed a hard outer crust which seemed to prevent fluid loss and bacterial penetration. None of the grafts became infected and it may be pertinent that decreased bacterial counts are found in experimentally infected burn wounds in rats dressed with a bovine skin collagen preparation[11].

Thus, it might prove possible to use dermal collagen allografts for the permanent replacement of lost or damaged skin in humans as well as animals. The long term fate of such grafts is currently under investigation.

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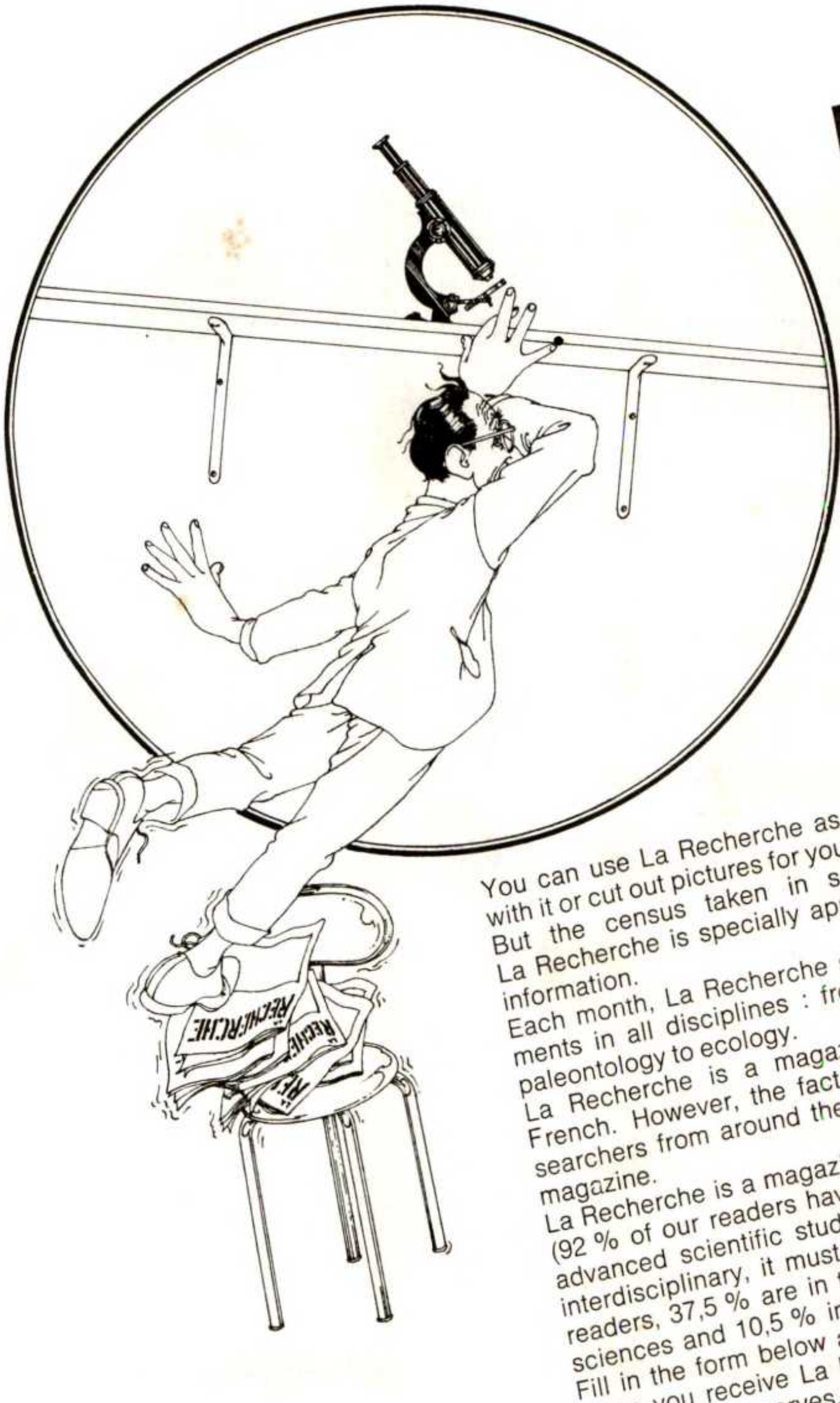

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Melanoma cells which require cyclic AMP for growth

THE cyclic forms of AMP, GMP and CMP have been implicated as agents which either stimulate or inhibit cell division. Pastan *et al.* reviewed this topic[1] and others have dealt with it[2–6]. Our studies of the growth of cultured melanoma cells have focused on the inhibitory effects of cyclic AMP[7]. We analysed variant cells that can grow in the presence of agents, such as melanocyte stimulating hormone (MSH), which increase the intracellular levels of cyclic AMP and inhibit the growth of wild-type cells. We describe here a class of variants which are not only resistant to the inhibition, but require cyclic AMP to grow at the rate normally observed in wild-type cells. In one such variant, division was markedly stimulated when either MSH, theophylline, isobutyl methyl xanthine, cholera toxin, prostaglandin E_1 (PGE_1) or dibutyryl cyclic AMP was added to the medium. Each agent inhibited the growth of wild-type cells. The cells were temperature-sensitive in that they expressed the variant phenotype at 37 °C, but behaved as the wild type at 40 °C. Studies of dose responses to dibutyryl cyclic AMP revealed that growth was also enhanced in the wild type by lower concentrations of the nucleotide (10^{-5} M), a fact that had gone unnoticed in melanoma cells but has been reported for other cell types[1]. These findings suggest a stimulatory as well as inhibitory role for cyclic AMP in control of growth of melanoma cells.

The wild-type cell line was a pigmented subclone of the Cloudman S91 melanoma which was selected for loss of hypoxanthine-guanine phosphoribosyl transferase activity[8]. This line was designated PS1-HGPRT-1. We exposed these cells to the mutagen N-methyl-N-nitro-N-nitrosoguanidine by standard procedures[9] and then selected for clones that were resistant to the inhibition of growth which occurs when MSH and theophylline are included in the culture medium. One clone, described below, required cyclic AMP for normal rate of growth and was designated PS1-HGPRT 1-$\mathrm{cA}^{\mathrm{dep}}$1. This clone will be referred to here as $\mathrm{cA}^{\mathrm{dep}}$.

Cells of $\mathrm{cA}^{\mathrm{dep}}$ grew poorly in normal F10 culture medium but had division rates similar to wild-type cells when the medium was supplemented with agents known to increase the intracellular levels of cyclic AMP (Fig 1*a*). In contrast, the growth of wild-type cells was inhibited by these agents (Fig. 1*b*). For example, in a typical experiment, the mean doubling time of $\mathrm{cA}^{\mathrm{dep}}$ cells was 96 h in normal F10 medium and 48 h in medium supplemented with MSH (2×10^{-7} M) and theophylline (2×10^{-3} M). For wild-type cells the doubling time was 41 h in normal F10 and 89 h in the supplemented medium. MSH or theophylline alone, dibutyryl cyclic AMP (2×10^{-4} M), PGE_1 (4×10^{-5} M), isobutyl methyl xanthine (1×10^{-4} M, data not shown) and cholera toxin (0.5 μg ml^{-1}, data not shown) each markedly stimulated the growth of $\mathrm{cA}^{\mathrm{dep}}$ cells and inhibited the growth of wild-type cells. The $\mathrm{cA}^{\mathrm{dep}}$ cells were temperature sensitive for the expression of the variant phenotype. They behaved as wild-type cells when cultured at 40 °C (Table 1).

Studies of dose-response to dibutyryl cyclic AMP showed that division by wild-type cells was maximally stimulated by a concentration of about 10^{-5} M. This was ten times

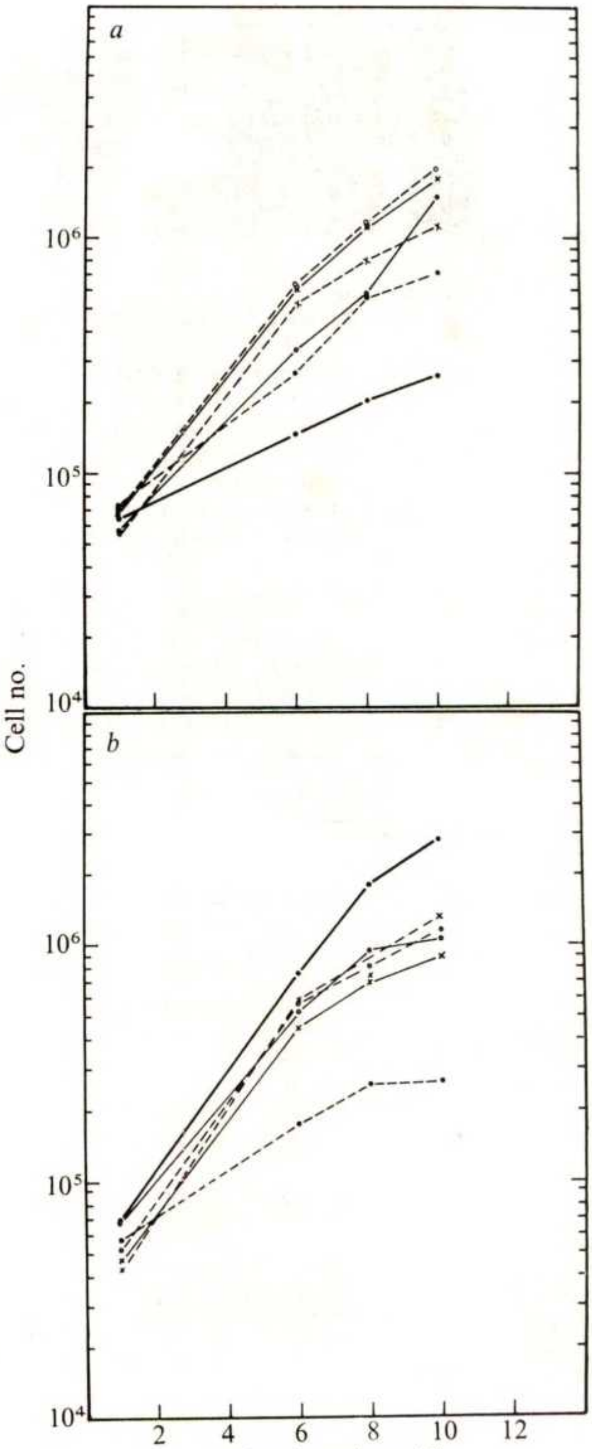

Fig. 1 Growth of $\mathrm{cA}^{\mathrm{dep}}$ (*a*) and wild-type cells (*b*) with various additives in the culture medium (see text). Cells (5×10^4) were inoculated into 4 ml F10 culture medium supplemented with 2% foetal calf serum and 10% horse serum in 30-ml Falcon tissue culture flasks. The temperature was maintained at 37 °C. Growth curves were constructed by counting cells from triplicate culture flasks in a Coulter counter. Medium was changed at 3-d intervals. Prostaglandin E_1 was given by Dr John E. Pike (Upjohn). ●—●, Control; ×— —×, PGE_1; ○— —○, dibutyryl cyclic AMP; ○—○, theophylline; ×—×, MSH; ●— —●, MSH and theophylline.

Table 1 Doubling times of cA^{dep} cells at 37 and 40 °C

	Length of cell cycle (h)	
	37 °C	40 °C
Normal medium	62	38
Medium + MSH	43	64

Cells (5×10^4) were cultured for 7 d in normal medium or medium containing MSH (2×10^{-7} M). Cultures in triplicate were then collected and counted in a Coulter counter. These experiments were repeated three times with similar results each time. Growth of wild-type cells was inhibited by MSH at both temperatures (data not shown).

lower than that for optimal stimulation of cA^{dep} cells (Table 2). As would be expected, we encountered some variability in the dose–response studies but the tenfold difference in optimal concentration remained constant. That the cells lacked HGPRT was not related to this phenomenon since wild-type cells that had normal levels of HGPRT were also stimulated by dibutyryl cyclic AMP (data not shown). It should also be noted that cA^{dep} and wild-type cells were always inhibited in growth by concentrations of dibutyryl cyclic AMP of 8×10^{-4} M or higher.

There has been some question about the specificity of the stimulation of growth by low concentrations of dibutyryl cyclic AMP since other compounds such as adenosine can elicit the same response (see for example, refs 1 and 10). We saw the stimulation, however, only with the cyclic forms of AMP. In an experiment parallel to that in Table 2, 5′-AMP, 3′,5′-cyclic GMP and its dibutyryl derivative were added to wild-type and cA^{dep} cells in concentrations ranging from 1×10^{-6} M to 5×10^{-4} M, and none of these compounds affected the growth of the cells. We conclude that the stimulation of growth of wild-type cells by the lower concentrations of dibutyryl cyclic AMP is a specific effect of the cyclic nucleotide.

Table 2 Doubling times of wild-type and variant cells at various concentrations of dibutyryl cyclic AMP

Molarity of dibutyryl cyclic AMP in culture medium	Length of cell cycle (h)	
	Wild-type cells	cA^{dep} cells
0	56	80
1×10^{-6}	34	58
4×10^{-6}	35	50
1×10^{-4}	39	42
2×10^{-4}	42	43
4×10^{-4}	53	53
8×10^{-4}	80	120

Cells (5×10^4) were inoculated into 4 ml F10 culture medium supplemented with 2% foetal calf serum and 10% horse serum in 30 ml Falcon tissue culture flasks. After 24 h the medium was changed and dibutyryl cyclic AMP was added at the concentration indicated. Growth curves were constructed by counting cells from triplicate culture flasks in a Coulter counter during 11 d. Medium was changed at 3-d intervals.

Reports from many laboratories have shown that cyclic AMP can inhibit the proliferation of various cell types. Far fewer reports have indicated that cyclic AMP has a mitogenic action as well (for example, refs 1 and 4). For example, it has recently been shown that DNA synthesis commences in normal rat livers when the animals are injected with dibutyryl cyclic AMP and theophylline[2]. Also, cyclic AMP levels increase after partial hepatectomy and remain high during the prereplicative period, suggesting that increased cyclic AMP levels signal the onset of liver regeneration[5]. Cyclic AMP may also trigger cell division in lymphocytes[6]. The temperature-sensitive cA^{dep} variant described here strengthens the possibility that cyclic AMP produces both kinds of effect physiologically in melanoma cells. Analyses of this variant should be useful for dissecting the mechanisms, and we are investigating the molecular intermediates involved. It will be interesting to determine if cyclic AMP-dependent variants exist among other cell types. Such variants could provide evidence for a generalised dual role of cyclic AMP in the control of cell proliferation.

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Molecular structure of nicotinamide adenine dinucleotide

NICOTINAMIDE adenine dinucleotide (NAD) has a fundamental role in metabolic processes as an electron transport molecule. Although its chemical structure was elucidated[1] in 1934, its detailed conformation remains still to be established in spite of numerous physicochemical applications[2]. NAD analogues with a variety of substitutions on the bases are known to retain considerable activity of the natural coenzyme as long as the pyrophosphate diester group has been retained[3,4]. The geometry of this backbone moiety is therefore indispensable to our understanding of the conformation and function of the coenzyme. We have so far no experimental evidence on this in NAD or any other nucleotide coenzyme molecule. X-ray studies have been possible only on those analogues[5,6] where the nicotinamide and adenine rings are linked by a trimethylene bridge. The results are conflicting and it is difficult to use them to provide a structural basis for the NAD molecule itself, particularly as the phosphate backbone is absent from these analogues.

We have determined[7] the molecular structures of cytidine-5′-diphospho-choline (CDP-choline) and CDP-5′. The study provides for the first time the detailed geometry of the pyrophosphate diester linkage present in a nucleotide coenzyme as well as its orientation with respect to the ribose moiety. The study shows further that the observed geometry is fully compatible with ester linkages to nucleo-

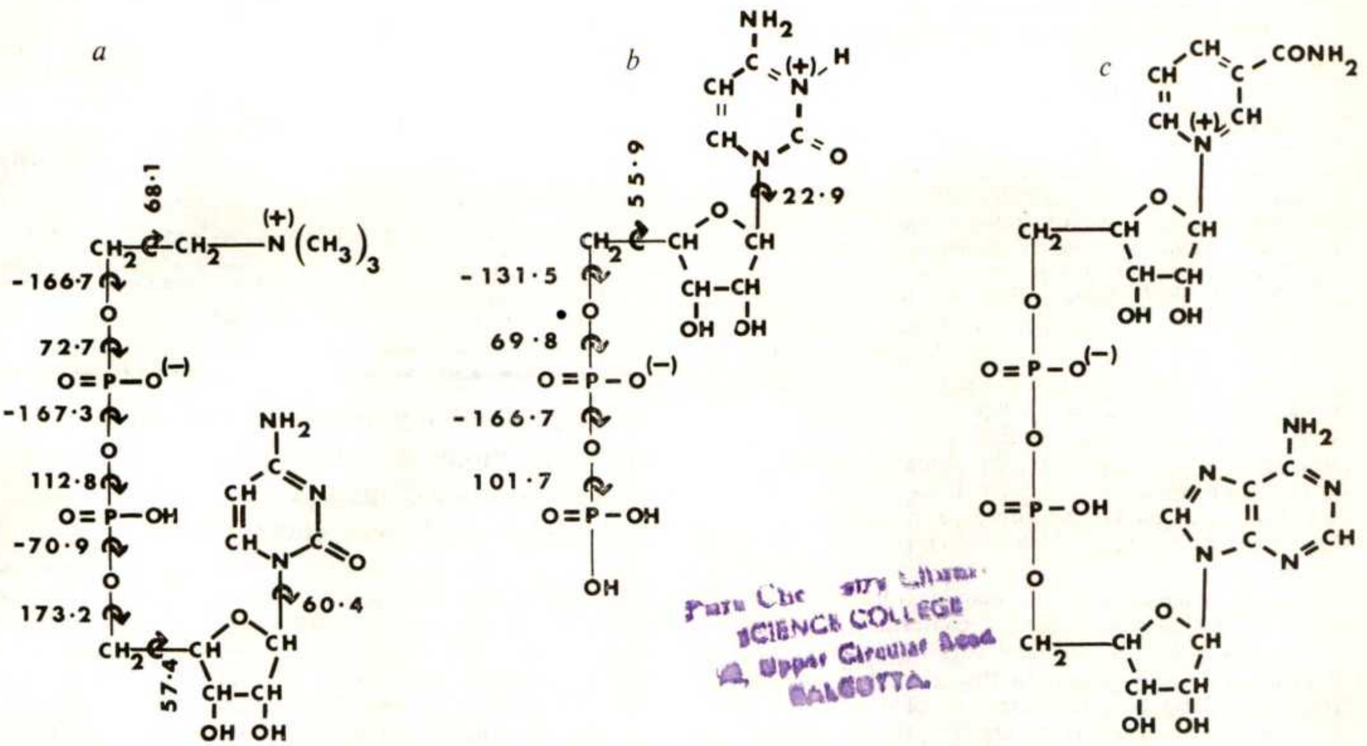

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Fig. 1 Chemical structures of CDP-choline (*a*), CDP (*b*) and NAD (*c*). Torsional angles (conventions as in reference 11) observed in the crystal structures are also shown. The geometry and orientation of the pyrophosphate group in CDP are those observed in CDP-choline from the choline end, that is, as if the choline moiety is replaced by cytidine. The diester thus has the optimal geometry required for ester links to nucleosides at each end, as required in NAD.

sides at both ends of the pyrophosphate. The chemical structures of these molecules along with experimental torsional angles are shown in Fig. 1.

The structure of NAD is also shown. There are a number of common features between the two nucleotide coenzymes CDP-choline and NAD. First, the diester pyrophosphate backbone of the dinucleotide is the same as that present in CDP-choline including a formal negative charge on a phosphate. One end of the bridge ester links to a nucleotide just as in NAD. The other end forms an

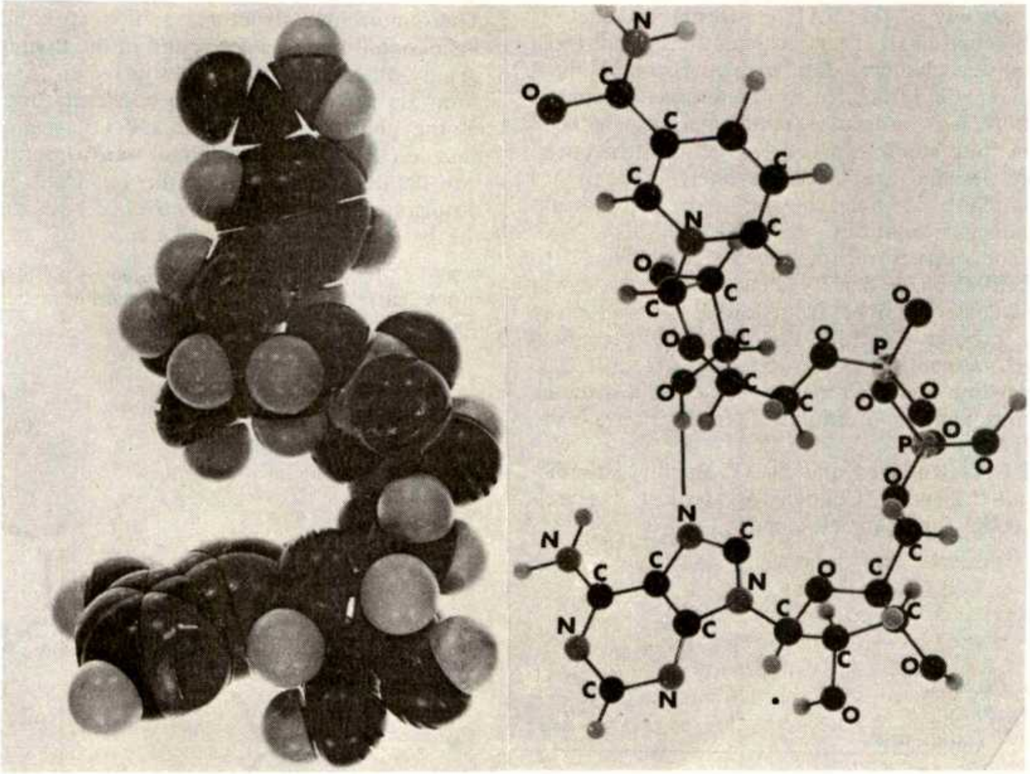

Fig. 2 Space filling and perspective views of the present NAD model.

ester bond with the choline moiety containing a quaternary nitrogen in CDP-choline whereas in NAD it is linked to the ribosyl nicotinamide moiety in which the nicotinamide is again present as a quaternary nitrogenous base. In CDP-choline the pyrophosphate is related to the nucleoside at one end and the choline moeity at the other by right- and left-handed (that is, opposite) rotations about the terminal ester bonds, respectively, as indicated by the sign of the torsion angles in Fig. 1*a*. Such a disposition of the pyrophosphate would also be expected if it were to link centrally two nucleosides instead of one nucleoside and one choline moiety. Examination of the various dihedral angles in CDP (Fig. 1) clearly demonstrates that its pyrophosphate geometry and orientation are simply those of CDP-choline, from the choline end the choline residue being replaced by the cytidine nucleoside. The pyrophosphate diester thus has the optimal geometry and conformation required for ester links to nucleosides at each end as in NAD and affords a reasonable basis for the proposed structure of the NAD molecule shown in Fig. 2.

Both the adenine and nicotinamide nucleosides of the model possess a *gauche–gauche* conformation about the C4′–C5′ bond. This is consistent with the inherent stability of nucleotides with respect to this geometry[8] as observed from X-ray data of nucleoside-5′-phophates, dinucleosides and other nucleic acid fragments. The rigidity of this conformation is maintained in the presence of pyrophosphate and even triphosphate linkages as observed in the crystal structures of CDP[7], CDP choline[7], UDP (M.A.V., M. V. Hosur, M. L. Post and O. Kennard, unpublished), and ATP[9]. The adenine and nicotinamide residues in the proposed structure of NAD are too far apart for either internal stacking or hydrogen bonding. Note that in none of the structures of NAD analogues so far solved by X-ray methods[5,6] or even in the cocrystallised salt of 1-methyl nicotinamide and aden-9-yl acetate[10] is there any complimentary or mutual hydrogen bonding between the nicotinamide and adenine moieties in spite of extensive hydrogen bonding found in the crystal structures.

The detailed nature of the model proposed here may facilitate further study of the NAD conformation, such as theoretical conformational energy calculations, and fresh assessment of physicochemical data such as those obtained from proton magnetic resonance and fluorescence studies of aqueous solutions. Molecular conformations deduced from crystalline state studies possess energetically favoured torsional geometries and are therefore likely to be maintained as dominant if not as the only equilibrium structures in aqueous solutions. Adams *et al.*[2] have proposed an extended structure for the NAD molecule to account for their electron density distributions of dogfish lactate dehydrogenase obtained from low resolution X-ray studies. In the present structure the diribotide pyrophosphate is folded instead of being extended. It still has, however, the adenine and nicotinamide bases at a distance of 10 Å and thus enables a similar interpretation of the electron density maps.

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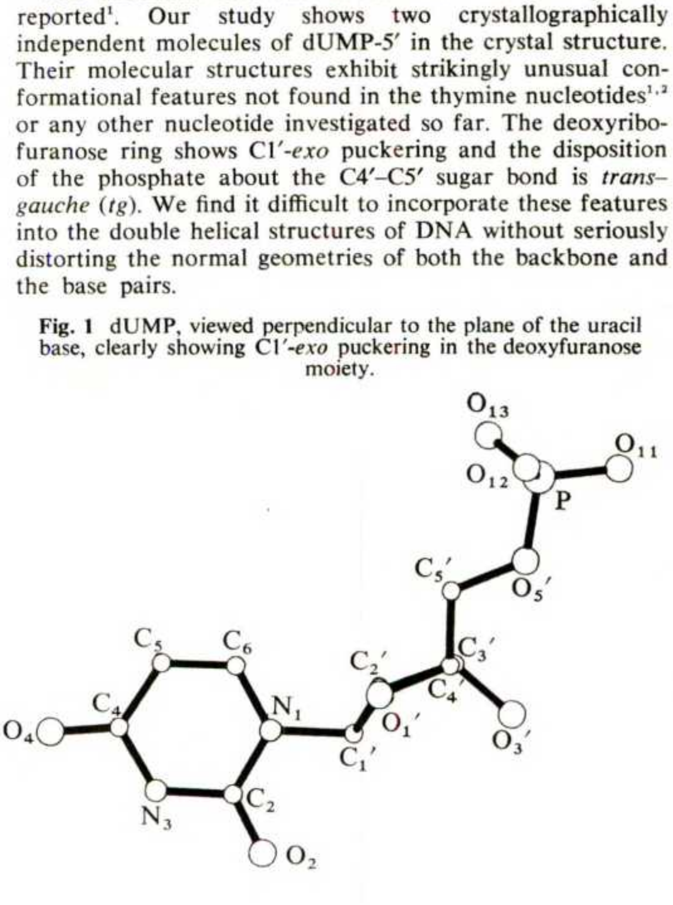
An uncommon nucleotide conformation shown by molecular structure of deoxyuridine-5′-phosphate and nucleic acid stereochemistry

CRYSTAL structure determinations of nucleic acid fragments have shown that several of the conformational features found in the monomeric building blocks are also manifested at the nucleic acid level. Stereochemical variations between thymine and uracil nucleotides are therefore of interest as they can provide a structural basis for some of the differences between the conformations of DNA and RNA. X-ray studies have so far not shown any major dissimilarities between these two nucleotide species although the sugar ring of deoxyribonucleotides is found to possess greater flexibility than that in ribonucleotides. We report here the molecular structure of deoxyuridine-5′-phosphate (dUMP-5′) which is not a common monomer unit of DNAs as it is replaced by its 5-methyl analogue deoxythymidine-5′-phosphate (dTMP-5′). The investigation was undertaken to help determine whether or not this implied a fundamental difference between the geometries of these two molecules.

The molecular structure of dTMP-5′ has already been reported[1]. Our study shows two crystallographically independent molecules of dUMP-5′ in the crystal structure. Their molecular structures exhibit strikingly unusual conformational features not found in the thymine nucleotides[1,2] or any other nucleotide investigated so far. The deoxyribofuranose ring shows C1′-*exo* puckering and the disposition of the phosphate about the C4′–C5′ sugar bond is *trans–gauche* (*tg*). We find it difficult to incorporate these features into the double helical structures of DNA without seriously distorting the normal geometries of both the backbone and the base pairs.

Fig. 1 dUMP, viewed perpendicular to the plane of the uracil base, clearly showing C1′-*exo* puckering in the deoxyfuranose moiety.



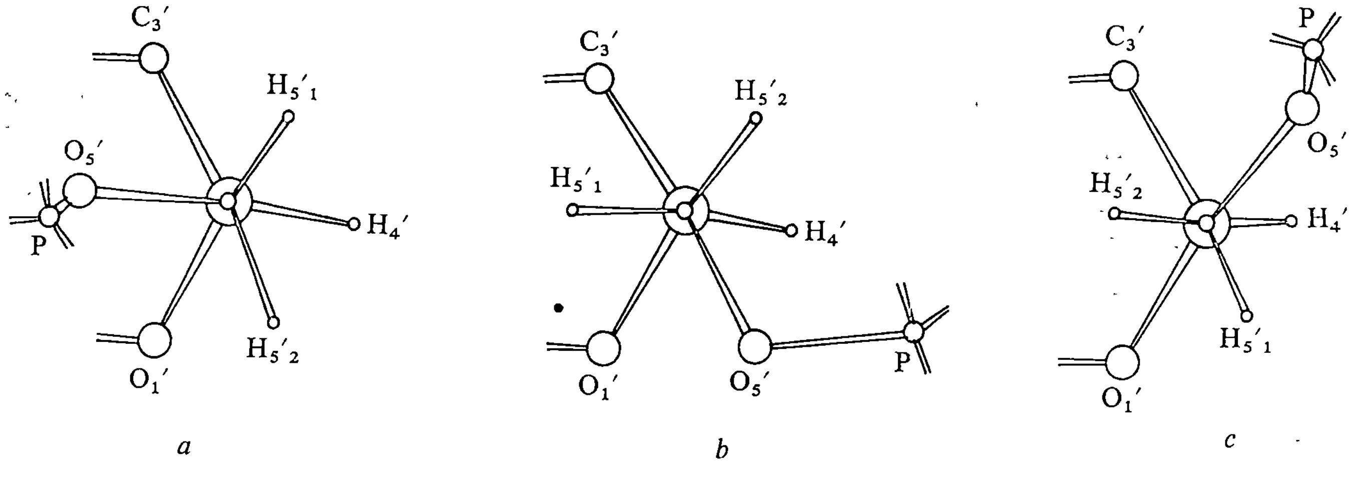


Fig. 2 The three possible staggered conformations of the phosphate moiety in nucleotides. Views, down the C5'–C4' bond, show the relevant region of: *a*, dTMP[1] (*gg*); *b*, dGMP[9,10] (*gt*); *c*, dUMP (*tg*).

dUMP-5' was crystallised[3] as a disodium salt from water–acetone solutions in the form of flat plates with monoclinic symmetry, space group $P2_1$ and cell constants $a=7.25$, $b=35.45$, $c=7.13$ Å, $\beta=102.2°$. The measured density of 1.68 g cm^{-3} ml^{-1} was found consistent with the presence of two independent nucleotide molecules in the asymmetric unit with five waters of hydration per nucleotide. X-ray data were collected photographically using CuKα radiation and the intensities were estimated by visual comparison with a calibrated graded intensity scale. The location of the phosphorus vectors was not straightforward because of heavy overlap of peaks in the Harker section. The structure was solved by a judicious combination of Patterson superposition maps, direct methods and difference Fourier synthesis. It was refined by full matrix least square methods to a value of 8.9% for the conventional *R* factor. Where possible, the hydrogen atoms of the nucleotide molecules were fixed using their expected geometry.

The asymmetric part of the unit cell contains two crystallographically non-equivalent molecules of the nucleotide. The bond lengths and angles within them are consistent and comparable to those in other uracil nucleotides[4,5]. Full details of the molecular dimensions and structure solution will be published elsewhere. The two independent molecules show remarkable conformational consistency, the value of the dihedral angle O1'-C1'-N1-C6 being equal to 57.1 and 59.9° for the two molecules. The orientation of the uracil base with respect to the sugar ring is therefore *anti*, the only conformation about the glycosidic bond to be observed so far in crystal structures of nucleotides (for notations see ref. 6).

The best four atom least squares plane for the deoxyribose ring in molecule B passes through O1', C4', C3' and C2' atoms. The C1' atom shows maximum deviation from this plane of 0.502 Å, on the side opposite the C5' atom, compared with 0.017 Å shown by the C2' atom. The sugar ring therefore has the C1'-*exo* envelope conformation, clearly seen in Fig. 1. The sugar conformation in molecule A can be described C2'-*endo*–C1'-*exo* as seen from the torsional angles given in Table 1. Those about the exocyclic C4'–C5' sugar bond for the two molecules are: O5'–C5'–C4'–C3' = −64.7 and −65.9°, O5'–C5'–C4'–O1' = 171.1 and 172.2°. The conformation of deoxyuridylic acid in both the independent molecules is therefore *tg* about the C4'–C5' bond. Figure 2 illustrates this geometry along with the other two staggered orientations about this bond, *gauche-gauche* (*gg*) and *gauche–trans* (*gt*)

Thus in having the *anti* conformation about the glyco-

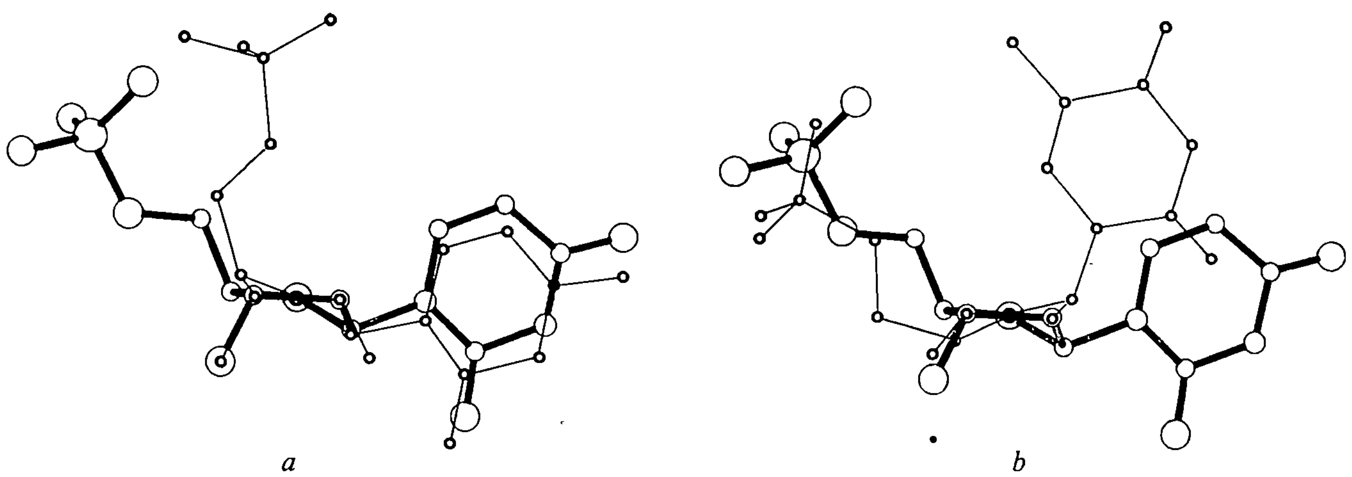


Fig. 3 Views of *a*, 5'-UMP (ref. 5) and *b*, dTMP[1] superimposed on dUMP (shown in heavy lines), to indicate the disturbance in overall molecular spatial character caused by *tg* and C1'-*exo* conformation. In each case the view is taken down the bisector of the C2'–O1'–C3' angle.

Table 1 Torsional angles

	Molecule A	Molecule B
C4′–O1′–C1′–C2′	−24.1°	−31.4°
O1′–C1′–C2′–C3′	30.8°	33.3°
C1′–C2′–C3′–C4′	−23.4°	−22.2°
C2′–C3′–C4′–O1′	10.3°	5.3°
C3′–C4′–O1′–C1′	8.9°	16.1°

sidic linkage, dUMP-5′ is similar to dTMP-5′, UMP-5′ and all other nucleotides in the solid state. Its other two characteristics, the C1′-*exo* puckering of sugar and the *tg* conformation about the C4′–C5′ bond, are fundamentally different from those of all the other nucleotides investigated so far. The most commonly observed conformation has been *gg* (Fig. 2*a*), whereas the *gt* conformer (Fig. 2*b*) has been observed in only two nucleotides, dGMP-5′ (refs 9 and 10) and 6-azauridine-5′-phosphate[11] which is not a normal nucleic acid component. The refined models[12] of double helical DNA and RNA also possess the *gg* conformation about the C4′–C5′ bond and C2′/C3′ *endo* modes of puckering for the sugar. If the dUMP conformities were to be inserted into these structures, we suggest that this would considerably distort the normal stacking and hydrogen bond formation between the bases as well as the geometry of the backbone chain, thus exerting a destabilising influence on the double helix. The difficulty of incorporating *tg* (g^-) conformation around the C4′–C5′ bond into helical structures has also been realised in an earlier study[6]. It does seem, therefore, that the non-occurrence of this nucleotide in DNAs has a structural basis. The nature of changes brought about by the insertion of the dUMP conformation in the double helical structure may be seen in Fig. 3 which shows two perspective views of the dUMP structure superposed on the structure of UMP and dTMP.

There has been considerable interest in the study of the conformational freedom allowed in nucleic acids and their constituents. Based on careful surveys of crystal structure data, Sundaralingam[7] has proposed that the nucleotide unit is essentially rigid, having a *gg* conformation about the C4′–C5′ bond, and that the flexibility of polynucleotide chains is found mainly about the internucleotide P–O linkages. He has also envisaged alternative conformation for nucleotides generally correlated with less favoured sugar conformations[8]. Structural evidence on dGMP-5′ (refs 9 and 10) and our results demonstrate the validity of this and establishes that significant flexibility about the exocyclic C4′–C5′ sugar bond can exist, particularly when the sugar pucker is not restricted to the C2′ and C3′ atoms only. Although outside the preferred conformations found in nucleotides, these stereochemical features seem to be sufficiently relevant to nucleic acid structures, particularly when one has to consider the influence of local distortions in the double helix on the tertiary structure of DNA. Crick and Klug[13] have made specific use of this property in their model for the highly folded structure of DNA in chromatin They surmise that these foldings could be brought about by localised kinks in the double helical structure of DNA, where the backbone conformation is *gt* instead of *gg* about the C4′–C5′ bond Such a stereochemistry enables bending of the double helix while maintaining the integrity of the base pairs. In contrast, the *tg* conformation about the C4′–C5′ bond together with the C1′-*exo* puckering of the sugar as found in the present structure, tend to disrupt the normal stacking and base-pairing geometry; and thus may be appropriate local conformations of DNA where separation and unwinding of the strands is presumably involved, such as during replication.

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Human endonuclease activity for DNA apurinic sites

APURINIC sites in cellular DNA may occur secondary to the slow hydrolysis of purines in physiological conditions[1] or the rapid hydrolysis of alkylated purines[2]. Endonucleases which incise double-stranded DNA at apurinic sites have been purified from *E. coli*[3], calf thymus[4] and rat liver[5]. It has been proposed that such endonucleases hydrolyse the apurinic site as the first step of a repair process similar to excision-repair of ultraviolet photoproducts[6]. Lindahl and Nyberg calculated that spontaneous depurination, if unrepaired in postmitotic cells such as human neurones, would result in the loss of 3% of the total purines during a lifetime[1]. Such a loss could be sufficient to produce abnormal proteins which appear in ageing cells[7]. The more rapid depurination of alkylated bases could be related to the mutagenic and carcinogenic properties of alkylating agents[8]. Therefore, to relate the repair of apurinic sites to the pathogenesis of human disease, we have characterised the properties of apurinic site endonuclease activity in crude extracts of HeLa cells and then measured such activity in WI-38 cells and human skin fibroblasts We then compared enzyme activity in skin fibroblasts from individuals with two diseases in which repair of apurinic sites might be defective —progeria, characterised by premature ageing[9] and associated with abnormal thermolability of cellular enzymes[10], and Fanconi's anaemia, associated with an increased incidence of malignancy and *in vitro* susceptibility to chromosome breakage by alkylating agents[11].

We found apurinic site endonuclease activity in all human cell lines tested and no deficiency in progeria or Fanconi's anaemia cells. Apurinic sites were introduced into circular PM II phage DNA by heating at acid *p*H[12] and apurinic site endonuclease activity was assayed by measuring the conversion of super-helical DNA to the nicked form.

^{3}H-thymidine-labelled PM II DNA was prepared and HeLa cells were grown as before[13]. WI-38 cells were obtained through the courtesy of Dr David Sabatini (New York University Medical Center). Both lines were determined to be free of *Mycoplasma* by uridine phosphorylase assay[14]. *Mycoplasma*-free normal skin fibroblasts (CRL-1295), progeria skin fibroblasts (CRL-1277) and

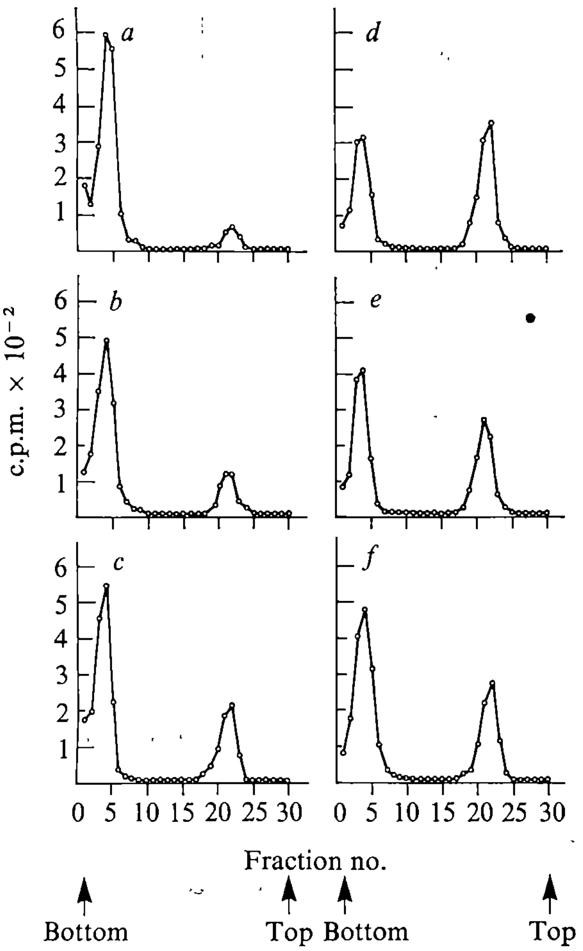


Fig. 1 Production of apurinic sites in PM II DNA. Aliquots of ^{3}H-thymidine-labelled PM II DNA in 0.1 M NaCl, 0.02 M Tris-HCl, *p*H 7.5, and 0.001 M EDTA were brought to *p*H 5.5 by addition of 0.1 M NaCl 0 1 M sodium citrate, *p*H 5.0 and 0.001M EDTA, and heated at 70 °C in a water bath for the times indicated. The DNA solution was then neutralised to *p*H 7.2 by addition of 0.2 M Tris-HCl, *p*H 8.0 and 0.001 M EDTA. To ensure hydrolysis of all heat-induced apurinic sites, the DNA was then incubated at room temperature for 4 h in 1.0 M glycine-NaOH, *p*H 12 8 (ref. 12). This solution was then layered on top of a 4.0 ml CsCl gradient, density 1 50, *p*H 13.0, and 0.02% sodium dodecylsulphate that had been formed by centrifugation for 60 min at 20 °C at 37,000 r p.m in an SW 50.1 rotor in an ultracentrifuge (Beckman L2-65B). The gradients were then spun for a further 105 min at the same speed. Gradients were collected by the method of Carrier and Setlow[24] and counted in toluene POPOP. The direction of sedimentation is from right to left. At least 90% of the applied radioactivity was recovered and all of it was under the two peaks. The fraction of superhelical and nicked molecules was determined by summing the radioactivity under each peak. Breaks per PM II molecule were calculated from the Poisson distribution which yields the formula: No. of breaks = $-lnx$ where x is the fraction of superhelical molecules[25]. All apurinic sites were hydrolysed under these conditions[12] so that each break represents one hydrolysed apurinic site. *a*, control; *b*, 2.5 min; *c*, 5 min, *d*, 15 min; *e*, 10 min, *f*, 7.5 min.

Fanconi's anaemia skin fibroblasts (CRL-1196) were obtained from the American Type Culture Collection. All fibroblasts and WI-38 were grown as previously described[13], with the exception of progeria fibroblasts which were supplemented with 20% foetal calf serum. WI-38 cells grew to 3-3 5×10^6 cells per 90-mm plastic dish, Fanconi's anaemia and normal skin fibroblasts to 2×10^6 cells per dish and progeria fibroblasts reached confluence at 10^6 cells per dish. WI-38 cells were received and assayed in passage 20-25. Fibroblasts were received frozen in early passage and assayed within 5-10 subsequent doublings. Cells were prepared for assay by the method of Brent[15] at 10×10^6–20×10^6 cells per ml. Protein was estimated by the method of Lowry *et al.*[16].

Figure 1 shows the production of apurinic sites in PM II DNA. Each minute of heating produced 0.04 apurinic sites per molecule in the conditions specified. Figure 2*a* shows that the introduction of 0.6 apurinic sites per molecule did not produce breaks in the DNA. Figure 2*b* shows that the

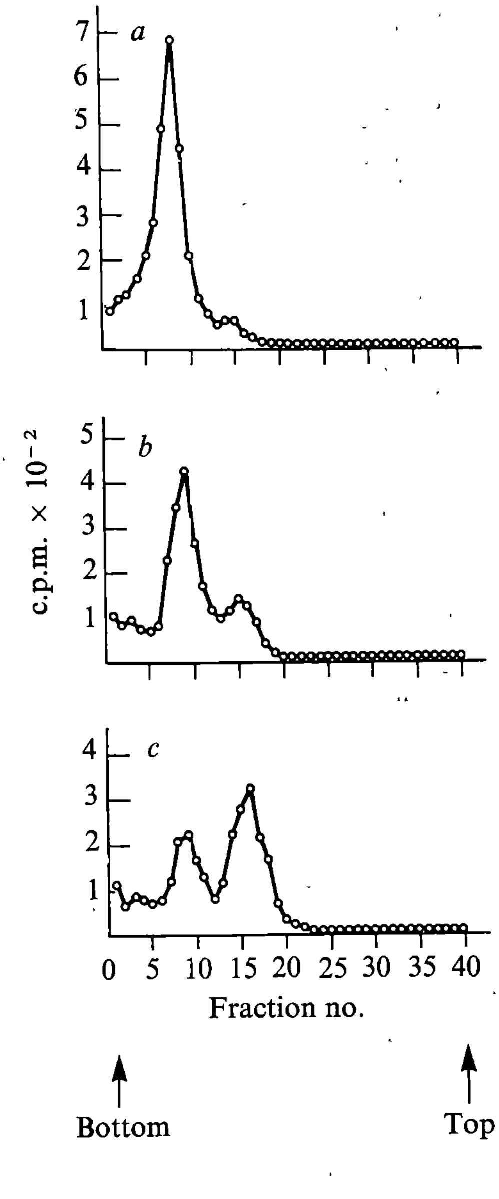


Fig. 2 Demonstration of apurinic site endonuclease activity in HeLa cell extracts. Aliquots of heated and subsequently neutralised DNA (0 3 μg) were incubated with HeLa extract of 100 μg protein (4 × 10^6 cells) for 30 min at 37 °C in a final volume of 120 μl. Conditions were identical to those described for ultraviolet endonuclease assay[13]. The reaction was stopped by chilling in ice and the entire mixture was then layered on to a 3.0 ml CsCl solution, density 1.50, *p*H 7.5, overlayed with light mineral oil and centrifuged in an SW 50.1 rotor at 43,000 r.p m. for 2 h 45 min at 20 °C in an ultracentrifuge (Beckman L2-65B). Gradients were collected by the method of Carrier and Setlow[24] and counted in toluene POPOP The direction of sedimentation is from right to left. More than 90% of the applied radioactivity was recovered and all of it was under the two peaks Calculation of breaks per molecule as in legend for Fig. 1. *a*, DNA containing 0 6 apurinic sites per molecule without HeLa extract. *b*, DNA containing 0.2 apurinic sites per molecule with HeLa extract. *c*, DNA containing 0.6 apurinic sites per molecule with HeLa extract

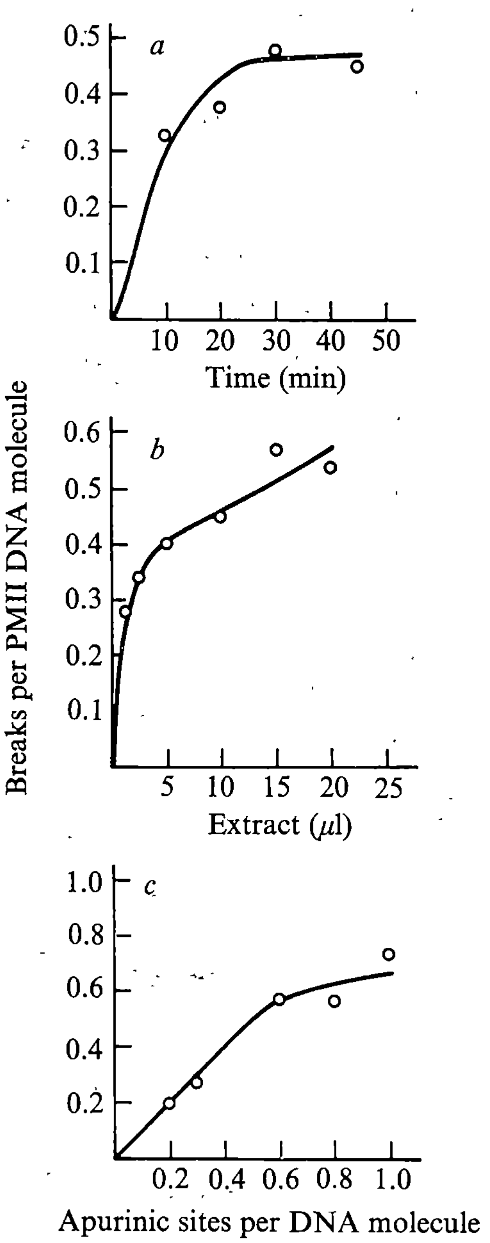


Fig. 3 Properties of HeLa apurinic site endonuclease activity. *a*, DNA (0.3 μg) containing 0.6 apurinic sites per molecule was incubated with 100 μg of protein of crude extract. At the times indicated, the reaction was stopped by chilling in ice and the mixture was analysed by centrifugation as in Fig. 2. *b*, DNA containing 0.6 apurinic sites per molecule was incubated with increasing amounts of HeLa extract for 30 min and analysed by centrifugation. Protein content was 4.6 mg ml^{-1}. *c*, Relation between the number of breaks produced by the enzyme activity and the number of apurinic sites on the DNA. Samples of DNA containing the number of apurinic sites indicated on the abscissa were incubated with 100 μg protein HeLa extract for 30 min and then analysed by centrifugation.

HeLa extract incubated with DNA containing 0.2 apurinic sites per molecule produced 0.2 breaks per molecule. Figure 2*c* shows that the HeLa extract produced 0.6 breaks per molecule when the DNA contained 0.6 apurinic sites per molecule.

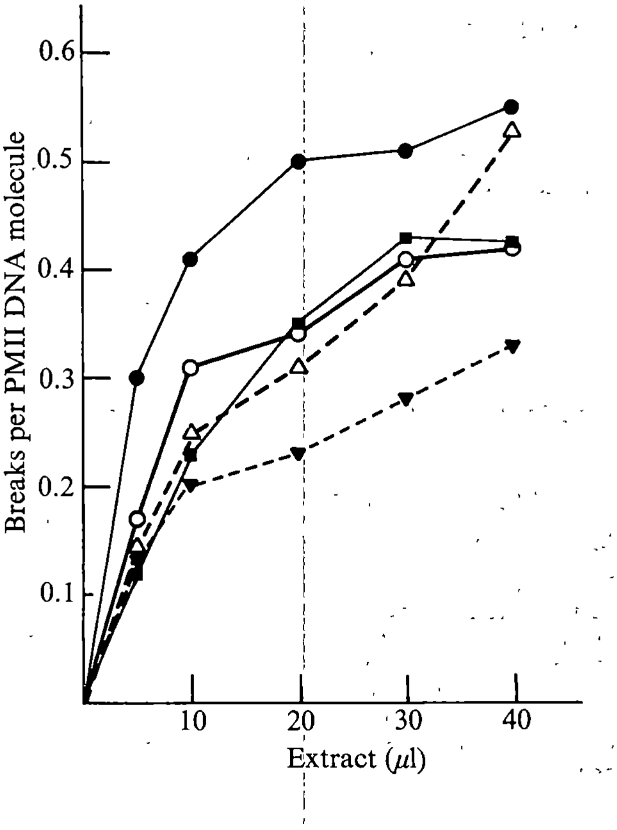
Figure 3 summarises the properties of the endonuclease activity in crude extracts of HeLa cells. Figure 3*a* shows that the reaction reached completion within 30 min. Figure 3*b* shows that activity increased with increasing protein content. An extract of 3×10^5 HeLa cells produced breaks at every apurinic site. Figure 3*c* shows that the activity in an extract of 3×10^5 cells produced breaks for every apurinic site in a 1:1 ratio up to 0.6 apurinic sites per molecule and then plateaus.

Figure 4 compares apurinic site endonuclease activity of extracts of different cell lines. There was no deficiency of activity in progeria or Fanconi's anaemia cells.

These results demonstrate endonuclease activity against apurinic sites in DNA in crude extracts of several human cell lines. Therefore, there are at least two putative human repair endonuclease activities: one directed against ultraviolet damage[13,15] and the other against apurinic sites. That these are distinct is suggested by the fact that apurinic site endonuclease activity is related to the number of alkali labile sites in DNA (Fig. 3*c*) while ultraviolet endonuclease activity is not[13,15]. Purification of enzymes from calf thymus has yielded an apurinic site endonuclease with virtually no activity against ultraviolet irradiated DNA[17], and an ultraviolet endonuclease with no activity against apurinic sites[18]. In contrast to our finding that ultraviolet endonuclease activity was much higher in HeLa and WI-38 than in primary skin fibroblasts[13], apurinic site endonuclease activities were more similar in the cell lines.

The role of this activity in human DNA repair is uncertain. Actual repair of apurinic sites *in vivo* cannot be measured since there is no method for specifically introducing such sites in cellular DNA[4]. But the widespread occurrence of apurinic site endonucleases suggests that such lesions are repaired by cells[19]. That apurinic site endo-

Fig. 4 Comparison of apurinic site endonuclease activities of cellular extracts. PM II DNA was heated to yield 0.6 apurinic sites per molecule. Cell extracts were prepared at a concentration of 10^7 cells per ml. After 30 min of incubation, the reaction mixture was analysed by neutral caesium chloride sedimentation velocity centrifugation. Results are calculated as previously. Unheated DNA was not broken by 40 μl of any of the cell extracts. ●, HeLa cells; ■, WI-38; ▼, normal skin fibroblasts (CRL-1295); ○, Fanconi's anaemia fibroblasts (CRL-1196); △, progeria fibroblasts (CRL-1277). Each point represents the result of an individual gradient except for CRL-1295 which are the average of three gradients.



nuclease activity is normal in progeria and Fanconi's anaemia cells indicates that either repair is normal, and that unrepaired apurinic sites do not have a role in the pathogenesis of these diseases, or repair might be abnormal due to a deficiency of other enzymes of the excision repair system. This possibility is suggested by a diminished rate of rejoining of single-stranded DNA breaks induced by ionising radiation in progeria cells[20] and a defect in exonucleolytic degradation of segments of DNA containing ultraviolet-induced pyrimidine dimers in Fanconi's anaemia[21]. It must be noted that the defect in progeria may be artefactual[22] and the defect in Fanconi's anaemia is evident only at high levels of irradiation[21]. Finally, there may still be a repair apurinic site endonuclease deficiency in progeria and/or Fanconi's anaemia, but as shown for ultraviolet sensitive mutants of *E. coli*, multiple endonucleases may be present in crude extracts and measurement of composite activity would not reveal deficiency of the actual repair enzyme[23]. Purification of endonucleases from human tissue and substrate characterisation is necessary to explain the role of these enzymes in the repair of genetic damage.

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IgM the secretory immunoglobulin of reptiles and amphibians

The major secretary immunoglobulin in mammals (IgA) has been studied extensively but there is little direct evidence about its phylogenetic origins (reviewed in ref. 1). The degree of sequence homology between the C terminal end of human α_1 and μ heavy (H) chains suggests that the α and μ chains diverged $\simeq$ 175 Myr ago, or at the time birds and mammals evolved from reptiles[2]. Several reports suggest the existence in birds of an Ig analogous to mammalian IgA[3,4]. We have therefore looked for a comparable Ig in reptiles and amphibia. We found that these lower vertebrates do have a primitive secretory Ig, but it is similar to serum IgM.

Most of this study was concerned with Ig in the garter snake (*Thamnophis ordinoides*). This species has three antigenically distinct serum Ig classes: a 20.5S macroglobulin (called IgM) and two $\simeq$9S gamma globulins, Ig-1 and Ig-2 (our work in preparation with D. Leong and L. Thomas). Antiserum to snake serum Ig (IgM, Ig-1 and Ig-2) was prepared in rabbits by injection of snake anti-HEA antibody eluted from an affinity column. This antiserum was made specific for Ig-1 by absorption with normal snake serum (details will be described later).

Rabbit antiserum to snake bile was produced as described in the legend of Fig. 1; antiserum obtained 3 weeks after inoculation reacted with one fast gamma and two alpha serum proteins. Radioimmunoelectrophoresis of serum from snakes injected with HEA revealed that the gamma precipitin line specifically bound HEA ^{131}I and fused in reaction of identity with the known IgM line developed with rabbit anti-snake Ig. Anti-bile antiserum was made specific for IgM by absorption with pooled anodal fractions from a Pevikon block electrophoresis[5] of normal snake serum. Even after two boosters of bile only trace amounts of antibody to other serum gamma globulins were seen. In contrast, rabbit antiserum to purified serum IgM contained abundant antibody cross reacting with common antigens on 9S–Ig (probably light (L) chain determinants) (our work in preparation with D. Leong and L. Thomas). IgM was detected in bile and succus entericus (SE) by gel diffusion, but neither anti-bile IgM nor anti-serum IgM detected any antigenic differences between serum and secretory IgM. Thus, using the available antisera, no evidence was obtained for the existence of an extra antigenic fragment on secretory IgM.

Sucrose density gradient analysis (Fig. 1) revealed distinct differences in molecular size between serum and secretory IgM. Serum IgM was detected in a sharp concentration peak (S $\simeq$20.5S) whereas the calculated S values for SE and bile IgM were more heterogeneous (IgM SE $\simeq$15S and 20.5S, IgM bile $\simeq$17S).

The IgM concentration in bile and SE, as determined by Mancini radial immunodiffusion[5], was 14–25% that of serum. (These figures are an overestimate because of differences in molecular size of serum and secretory IgM.) Although IgM was not secreted against a concentration gradient, the data indicate that the presence of this Ig in secretory fluids was the result of selective transport. That is, using gel diffusion, IgM was the only Ig detectable in bile and the predominant Ig in SE (which also contained small amounts of Ig-1 and Ig-2). The relative concentrations of IgM and Ig-1 in SE were determined by the Laurell antibody–Agarose technique using rabbit antisera monospecific for bile IgM or serum Ig-1. Assuming passive diffusion of IgM and Ig-1 from serum to SE, the relative amount of Ig-1 in SE (that is, relative to the serum concentration) should be greater than that of IgM because of the smaller molecular size of Ig-1 (9S) compared with IgM (21S). The concentration ratio of SE– serum for IgM was, however, 56 times greater than that of Ig-1. To test the possibility that the predominance of IgM in external secretions was due to selective degradation of 9S-Ig, serum was diluted 1 : 1 with bile, SE or phosphate-buffered saline and incubated at 24 °C for 48 h. Using Mancini radial immunodiffusion with rabbit anti-snake Ig incorporated into the agar, no change in the ring diameters of IgM (inner) or Ig-2 (outer) were detected even after 48 h incubation. Furthermore, twofold dilutions of serum kept at 4 °C were compared by immunoelectrophoresis with similar dilutions of serum incubated as above with secretory fluids to determine the dilution at which the Ig-2 precipitin line was undetectable. Both control and experimental dilutions had similar and distinct Ig-2 precipitin endpoints of 1 : 32. These findings suggest that the relative abundance of IgM in bile and SE was a function of selective transport across the mucosa and was not due to loss of 9S Ig from the secretory fluid by proteolysis.

Preliminary evidence indicates a similar secretory Ig system

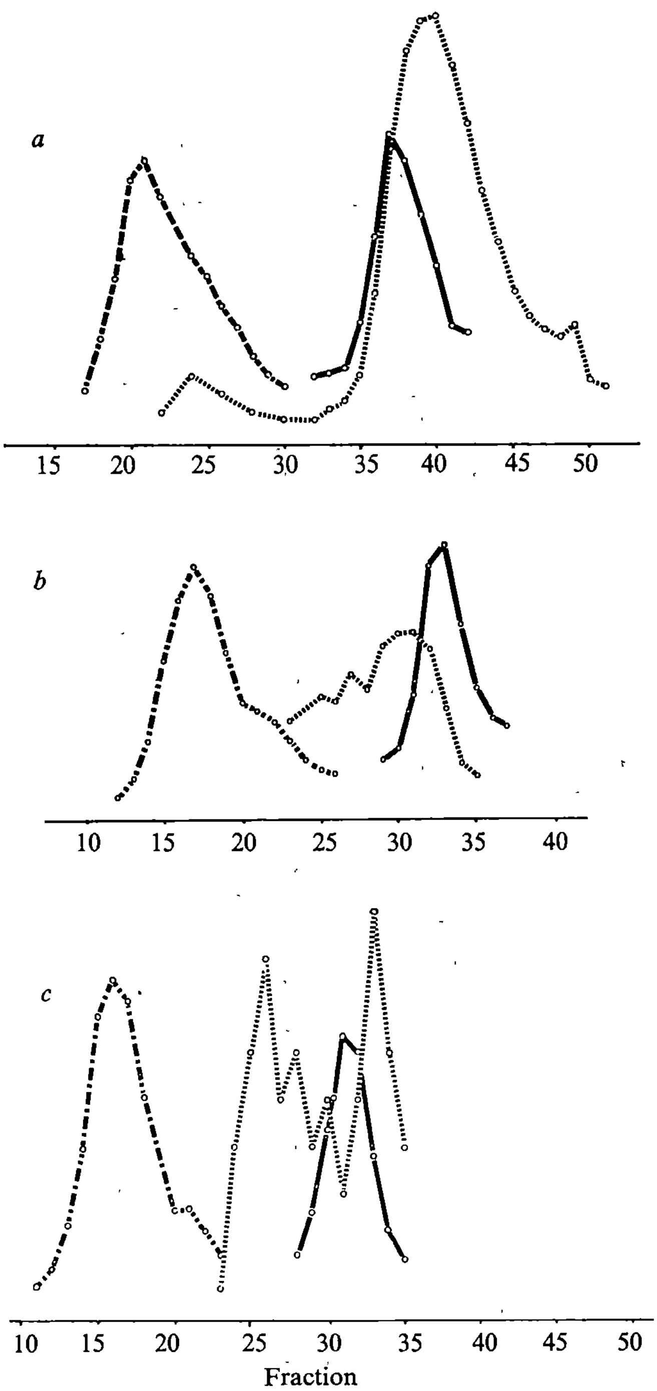

Fig. 1 Sucrose density gradient analysis of *a*, garter snake serum; *b*, bile; and *c*, succus entericus (SE). Bile was aspirated from the gall bladders of snakes and then pooled, dialysed and concentrated by negative pressure dialysis. SE was obtained from the intestines as described before[5]. 10–44% linear sucrose gradients were centrifuged for 18 h at 35,000 r.p.m. as described previously[5] using ^{125}I-labelled rabbit gamma globulin (— • —) and ^{131}I-labelled hog thyroglobulin (—) as 7S and 19S markers, respectively. The fraction number is represented on the abscissa, relative IgM concentration and c.p.m. on the ordinate. Fractions (0.1ml) were removed from the top of the gradients and analysed for IgM concentration by the Laurell antibody–Agarose technique[5] using specific rabbit anti-garter snake IgM, produced by injection of 200 µl of concentrated bile (equivalent to neat bile from two snakes) in an emulsion prepared with equal volumes of M/15 PBS and Freund's complete adjuvant (Difco). Because emulsification of bile was very difficult it was necessary to prepare the emulsion in a Sorvall Omni-Mixer using 10 ml of PBS and 10 ml of adjuvant Rabbits were injected subcutaneously at multiple sites and were boosted 3 and 6 weeks later with 100 µl of bile in incomplete Freund's adjuvant (Difco). After absorption (see text) the antiserum reacted only with IgM in serum or secretory fluids. The approximate S values of the IgM concentration peaks were: *a*, serum$\simeq$20.5S; *b*, bile$\simeq$17S; and *c*, SE$\simeq$15S and 20.5S. . . . . . , IgM.

in turtles (*Chrysemys picta*) and bullfrogs (*Rana catesbeiana*) because two rabbits immunised with neat bullfrog bile and two rabbits immunised with turtle bile each produced antisera which reacted specifically with their respective serum IgM. None of the antisera detected antigenic differences between serum and secretory IgM. It was particularly interesting that rabbit anti-snake, bullfrog and turtle bile revealed no cross reaction between the serum IgM of these species. In contrast, bullfrog anti-rabbit gamma globulin showed broad cross reactivity with other mammalian gamma globulins[6]. This phenomenon is discussed further in ref. 6.

It is noteworthy that the production of antisera specific (except for anti-α globulins) for snake, turtle or bullfrog IgM was achieved simply by injection of rabbits with bile from the respective species. Purified snake serum IgM, however, elicited abundant cross reacting (L chain) antibody. We are investigating whether these specificity differences resulted from unique properties of secretory IgM or from differences between the immunising emulsions (see legend to Fig. 1).

Sequence analysis of human Ig has revealed marked structural similarities between IgM and IgA[2,7]. These two Ig classes also resemble one another functionally, in that IgM can substitute for IgA in external secretions of patients with selective IgA deficiency[8]. The results of our study indicate that IgM served the function of a secretory Ig before the evolution of the α-heavy (H) chain gene. If sequence analysis of avian secretory Ig corroborates its homology with mammalian IgA, the lack of a unique class of secretory Ig in present day reptiles would place the evolvement of the α-H chain gene in a high reptilian ancestor of birds and mammals.

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Corrigendum

The complete authorship of the article "Age of KBS Tuff in Koobi Fora Formation, East Rudolph, Kenya" (*Nature*, **258**, 395; 1975) should be:

G. H. Curtis, R. E Drake, T. E. Cerling, B. L. Cerling and J H. Hampel.

Erratum

In the article "Post-transcription control of isocitrate lyase induction in the eukaryotic alga *Chlorella fusca*" by A. H. Scragg, P. C. L. John and C. F. Thurston (*Nature*, **257**, 498, 1975) the second line of text below the legend to Fig. 2 should read . . . concentration was largely isocitrate lyase. This, in turn, demonstrated . . . and not as printed.

matters arising

[illegible]
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Defensive stoning by baboons

COMMENTING that deliberate stone-throwing by baboons had not been previously recorded by professional field obervers, Hamilton *et al.*[1] described the behaviour from a colony of Chacma baboons (*Papio ursinus*) in South West Africa. This is not, however, confined to *P. ursinus*; I saw essentially similar behaviour from Anubis baboons (*P. anubis*) when on a short visit to Beldong Forestry Nursery in the Jebel Marra Massif of the Sudan in December 1962. The nursery is in a sparsely populated area, and at the time of the visit, had been in existence several years. As far as I am aware, baboons were not hunted locally and, from their general behaviour, they seemed to be partially habituated to the presence of the local population.

The stone-throwing incidents occurred while I was photographing on a steep hillside at about 1,800 m, some distance from the nursery where the vegetation had been cleared from derelict cultivation terraces. While I had been working across the hillside a troop of baboons, seemingly attracted by my presence, approached cautiously, stopping at intervals, until they were on the second and third terraces directly above me. After a short time, during which the troop showed some agitation, the largest male started dropping stones down the slope towards me, and was later joined by two, sometimes three, of the older baboons. The stones were taken from the broken terrace edge and cast with an underhand shovelling motion, as described by Hamilton *et al.*[1]. By repeated trials I found that more stones were rolled down when I was bent over the camera on its tripod than when I stood to one side of it, suggesting that the apparent threat came from the observer–camera–tripod complex. This received some support from the baboon behaviour when I finally tried to photograph them casting stones; when I took up the camera and tripod for a closer shot, the troop fled upslope with much barking, the majority going over the brow of the hill, though some of the largest animals lingered on the skyline when I did not follow.

I took no measurements of the rocks used by the baboons but my notes indicate a size similar to those used by *P. ursinus* (corrected data, see reply) and possibly somewhat larger, though in this case the selection would have been for smaller ones from the generally large rocks of the terrace wall. The 'aim' was uncomfortably good much of the time, but fortunately the terrace immediately above me caught or deflected most of the stones bouncing down the slope.

There is a difference between this example of stone-throwing and that of *P. ursinus*. In the latter, stones were cast from the rocky walls of the canyon where the baboons slept and retreated to when threatened by predators. In the present example, the behaviour took place well away from the sleeping area and the observer–camera–tripod complex seemed not to be perceived as a threat until the baboons themselves approached. As stressed by Hamilton *et al.*[1], partial habituation to humans was probably an important factor in this behavioural response. Although I have seen other baboons in the Sudan and northern Nigeria on inselbergs where stone-throwing might have been possible, I have seen no other example since. Elsewhere in the Sudan, the baboons were probably well accustomed to the presence of humans. In northern Nigeria generally, baboons are hunted and extremely wary, though in Yankari Game Reserve where, in contrast, they are protected and tame, they do not respond to camera and tripod in this manner.

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[1] Hamilton, W. J., Buskirk, R. E., and Buskirk, W. H., *Nature*, 256, 488 (1975).

HAMILTON *et al.* wish to draw attention to the following errors which appeared in their letter (*Nature*, **256**, 488; 1975): on page 489, line 9 should read 16.5 cm and 10.4 cm and not as printed; and in Table 1, last two lines should read SAM, subadult male and AM, adult male.

IN January 1973, I was conducting geological fieldwork in Baringo District. One afternoon I was approaching the fault scarp west of Lake Baringo, near Kampi-ya-Samaki, Kenya, with the intention of collecting some hyrax skulls which I had observed through binoculars when examining the nest of Verreaux's Eagle (*Aquila verreauxi*). As I approached the cliff, a troop of about 15 baboons near the cliff started climbing it. My presence had obviously alarmed them, and a few individuals were barking with the typical "wahoo" call. I continued to approach the cliff and was almost at the top of the scree slope when a rock flew past my head and splintered just below me. At first I thought a baboon had dislodged a stone accidentally, but when I looked up to see where the baboons were, I saw another deliberately extend his right arm over the edge of the cliff and release a second stone. This happened several more times, but as I was scrambling down the scree to get away, I neglected to make a note of exactly how many stones were tossed towards me. It was, however, probably no more than ten.

The baboons had been about 50 m from me, parallel to the cliff when I first encountered them, but they deliberately scaled the cliff and then walked along the top so as to be above me when they released the stones.

My experience differs somewhat from that reported by Hamilton *et al.*[1], in that this was the first time I had ever seen this troop of baboons, so the troop was in no way habituated to me. The whole episode, from initial contact to my departure, was over in less than 5 min. In other respects, however, the behaviour was as described by Hamilton *et al.*

This record extends the stoning behaviour to Kenya, and as postulated by Hamilton *et al.*, the behaviour may be quite widespread. My native field assistant assured me that he had seen it several times, and said that baboons will attack goats and kill and eat them. I have never seen the latter, but have been told by many Tugen people that it is a common occurrence there.

I question whether the stoning behaviour should be labelled "defensive". There were definite connotations of "offence" in the behaviour I saw. In particular, I was over 50 m away from the baboons at first contact, and it was they who approached me along the cliff top. When the baboons first saw me they were evidently agitated, but once on top of the cliff were out of danger. Only a couple of baboons tossed stones, and to do this they had to walk over 50 m towards me. The tossing was accompanied by rapid eyebrow raising and a tense alert carriage on the part of the thrower. All other baboons that I could see at the time were not unduly alarmed at my presence once they had reached the top of the cliff.

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

H–2 antigen restriction of viral infection

KOSZINOWSKI and Ertl demonstrated[1] that T lymphocytes immunised *in vivo* against virus kill virus-infected target cells only when these share H–2 antigens with the effector cells[2]. The commonly expressed view[3] is that the effector T cells recognise an antigen created by an interaction of H–2K or H–2D antigen molecules with viral protein.

To obtain further support for this notion, Koszinowski and Ertl show convincingly that anti-H–2 serum blocks killing when it is directed against the target cells, but not when directed against the effector cells. An unfortunate nomenclature (for example, anti-H–2^{kk} for (H–$2^{d/k}$ × H–2^{bb}) anti-H–2^{kk}) gives the impression that the sera are directed against the antigens of the whole *H–2* complex, when in fact both antisera used are directed only against the *K* end of the *H–2* complex. Thus the H–2D antigens of the effector cells are still free, and one cannot rule out the alternative hypothesis[1], that in order to kill, effector cells have to match up their SD antigens with those of the targets[4].

On the other hand, cytotoxicity is blocked completely whenever the antiserum, be it an anti-H–2K or an anti-vaccinia serum, is directed against the target cells, and this is taken as evidence for an interaction product being the target. It is important to know whether a similar blocking could be caused by any antiserum directed against the target cells, or only by anti-H–2 or anti-virus serum. Two points are of particular interest in this respect. First, blocking of cytotoxicity is complete although the antisera cover only the *K* end—if the blocking is as specific as in allograft reactions, where blocking the target antigens of only one end of *H–2* hardly affects the killing[5], this would mean that vaccinia, unlike LCM and ectromelia virus[2], modifies only the H–2K antigens. Second, one should note the blocking of F_1 target cells. For example, *H–2^k* effectors do not kill *H–2^d* targets, but they will kill $(d \times k)F_1$ as well as *H–2^k* target cells. Killing of *H–2^k* is blocked by anti-H–2K^k, not by anti-H–2^d, while killing of F_1 targets are blocked by both antisera. If the *H–2^k* effectors interact only with modified H–2^k antigens on the target cells, one should, again by analogy to the specificity in allograft reactions[6], not have expected anti-H–2^d serum to have any effect on the killing of F_1 target cells by *H–2^k* effector cells. My comments are based on the assumption that complete blocking is obtained only when all possible effector–target cell interactions are inhibited, and I have overinterpreted the data, if the relationship between percentage ^{51}Cr-release and number of lytic interactions is less quantitative.

The field of T-cell mediated immunity to virus infected target cells is fruitful and holds promise of information about the evolutionary significance of histocompatibility antigens[3]. It is therefore important, before we make conclusions about mechanisms, to know to what extent the phenomenon is analogous to allograft immunity.

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KOSZINOWSKI AND ERTL REPLY—We think that our data[1] do not allow the interpretation that serum mediated blocking of cell-mediated cytolysis (CMC) of virus infected syngeneic cells reflects an inhibition of all possible effector–target cell interactions. We cannot express this opinion since we find inhibitory activities also with antisera directed against vaccinia virus specific surface antigens[2].

The F_1 fibroblast targets were extremely sensitive for vaccinia infection which led to high spontaneous and small specific lysis. We are unsure whether the H–2^d inhibition in these experiments had a significance, since unspecific blocking occurred to some extent (see Fig. 1 of ref. 1). Anyway, xenogenic anti-target cell serum absorbed for H–2 had no specific inhibitory effect.

There are indeed indications that, in the experimental conditions we used the *K* end is the essential target. When we tested an anti-H–2D^k serum ((H–2^b × H–2^d) anti-K^d D^k) we found minimal blocking activities.

These results can be explained by two possible mechanisms. Vaccinia virus alters mainly *K*-end antigens so that these modified antigens can form a target for the T cell. On the other hand it is possible that T cells predominantly attack *K*-end alterations. Comparable results concerning different inhibitory activity on T-cell mediated killing by anti-*K*-end or *D*-end sera have recently been described in a tumour situation[3].

In the ectromelia system some mouse strains (B10.A (2R), B10.A (4R)) react more strongly at the *K* end[4]. Shearer *et al.*[5,6] describe effector cell activity against TNP-modified cells absolutely restricted to the *K* end in two mouse strains while other strains recognise the TNP-modified *D* end as well. Restriction of CMC can therefore be dependent on the target cell[5,6], the modifying agent and the mouse strain used, so that at present no generalised answer about *K*- or *D*-end specificity is possible.

We think that the intimacy hypothesis[7] of matching unaltered self antigens is unlikely. In addition to the arguments of Zinkernagel and Doherty[8] we found that the target antigen in the vaccinia infection for the CL has a close physical relationship to SD antigens[1]. Second, enzymatic reduction of H–2 antigens reduces CMC without affecting anti-vaccinia antibody mediated cytolysis (Ertl and Koszinowski, unpublished) and third, expression of the H–2 alloantigens is altered detectably by reduced serum absorbing capacity and inhibited allogenic CMC[9,10]. The latter observation especially, argues against the possibility of matching normal self antigens as a prerequisite for the recognition of new antigenic determinants.

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reviews

Falling bodies

The Mathematical Papers of Isaac Newton. Volume VI, 1684–1691. Edited by D. T. Whiteside, with assistance of M. A. Hoskin and A. Prag. Pp. xxxiv+614. (Cambridge University Press.) £25.00.

Newton by Vanderbank, Possession Royal Family

Mary Evans Picture Library

THE sixth volume in this definitive series devoted to Newton's mathematical papers covers the most important period in his scientific career, the climax of which was the publication of the *Principia* in 1687. Dr Whiteside has collected mathematically significant extracts from Newton's manuscripts bearing on his researches in the theory of moving bodies and of gravitation, which were his main concern at that time. These comprised the dynamical theory of the motion of bodies under central forces and his attempts on this basis to explain the observed motions of comets and the Moon.

In August 1684, following a challenge from Christopher Wren to produce an explanation of planetary motions, Edmund Halley went to Cambridge to discuss the problem with Newton. That momentous visit revived Newton's interest in the general dynamical theory of moving bodies which had lain dormant since his correspondence with Hooke nearly five years previously on the theory of falling bodies. In his final letter to Newton, Hooke raised the key question of the path of a planet acted on by the attraction of the Sun and, in particular, asked whether an inverse square law of force could make the planet deviate from its instantaneous uniform motion into an elliptical orbit with the Sun at a focus.

Three months after Halley had put the same question to him Newton sent to London the first version of a tract, in Latin, on the motion of bodies in an orbit. This has been reproduced by Dr Whiteside in the present volume, together with an English translation. It begins with a proof that Kepler's law of areas in generalised form can be deduced for a law of central force. Newton also showed that an elliptical path can be described under laws of force varying either as the distance or as the inverse square of the distance, the centres of force being, respectively, at the centre of the ellipse and at a focus. Among other results, he deduced for the latter case the third law of Kepler. He also went on to establish theorems concerning the motion of a projectile in a medium, resistance being directly proportional to speed.

On seeing this tract, Halley hastened again to see Newton in Cambridge. He found him already revising and enlarging the text into a full-scale treatise on motion. In the next two years, with Halley's encouragement, the text developed into, at first, two selected books, and ultimately the three that constituted the first edition of the *Principia*. The texts printed in the present volume illustrate the way in which the first book of the *Principia* evolved. Dr Whiteside has also included manuscripts concerned with particular problems such as that of the solid of least resistance to motion along its axis in a uniform fluid, the approximate determination of a parabolic cometary path, and the annual advance of the lunar apogee.

The volume concludes with some extracts from a scheme for revising the *Principia* in the early 1690s that Newton later abandoned.

The present volume maintains the high standards of annotation and printing set by previous volumes.

G. J. Whitrow

Enzyme kinetics

Kinetics of Enzyme Mechanisms. By J. Tze-Fei Wong. Pp. xii+294. (Academic: London and New York, August 1975.) £7.80; $20.25.

THIS book is written by a professional. Dr Wong has plenty of experience in analysing experimental data and he is fully aware that the sophistication of the algebraic treatment must be matched by the quality of the data.

The steady-state derivations of rate equations, which form an introduction, are rigorous and there are helpful tips even for those who have been teaching the subject for some time. Some of the nomenclature is unnecessarily different from the usual and some algebraic shortcuts may make the book difficult for the inexperienced. The author's critical judgement of different steady-state treatments will be very valuable for most of the potential readers. Dalziel coefficients are used with advantage. It is a pity, however, that Dalziel's treatment is not also used as an extended example for three substrate reactions. The warning about the caution necessary when playing ping-pong with Cleland (p.113) should be found in more treatments of enzyme kinetics.

Although it is good to see that the author has not completely neglected transient and relaxation kinetics, this area is not treated satisfactorily. Detail is given of the lag phase treatment developed by the reviewer 20 years ago. This has turned out to be of much less practical application than the subsequent analysis of the transient formation of enzyme bound products. The considerable amount of useful information obtained from this latter use of rapid reaction techniques deserves more space. Similarly the examples given for relaxation studies are those found in other text books. More recent and varied references would have been appreciated.

The above criticisms are obviously very personal ones and should not be taken too seriously. I would certainly recommend to any graduate student and research worker, who has some basic understanding of kinetics and calculus, to use this book for at least part of his journey towards a professional training in enzymology.

H. Gutfreund

Dangling infinitives

Writing Scientific Papers in English: An ELSE-Ciba Foundation Guide for Authors. By M. O'Connor and F. P. Woodford. Pp. 108. (Elsevier Scientific: Amsterdam, 1975.)

A PHYSICS student at my University attends about 1,500 hours of lectures, tutorials and laboratory classes on physics and mathematics during his undergraduate career. On completing his research a postgraduate must write a 30,000 word thesis, and may publish several articles in scientific journals. In spite of this, there is no formal tuition at all on how to write papers and submit them for publication. As far as I know the situation is similar in other universities and in other scientific disciplines. It is therefore not surprising that the scientific 'literature' is frequently turgid, dull, ungrammatical and sometimes incomprehensible. A recent review article—written by experienced scientists—contained the following: "... these spectra will be discussed at length, shortly ...", "... the fluorescence spectra does not ...". The word "only" was consistently misplaced: "... such a symmetry is only provided by the interstitial site ...".

These errors are relatively minor, and one knows what the authors are trying to say. But if the natives write like this, what hope is there for foreign scientists, who are compelled to use English to reach the international scientific community?

This is where *Writing Scientific Papers in English* comes in. Its title is a bit misleading, in that the book covers the whole process of publishing a research paper. The chapters are headed Planning, Preparing, Writing the First Draft, Revising, Refining, Typing, Submitting the Final Version, Responding to the Editor, and Correcting Proofs, the first Appendix summarises the book (Steps in Writing a Paper), Appendices Two to Four deal with Units and Abbreviations, and Appendix Five with Expressions to Avoid. Over the next two years a series of supplementary booklets will be published, dealing with the problems of particular language groups, and with the various disciplines of science.

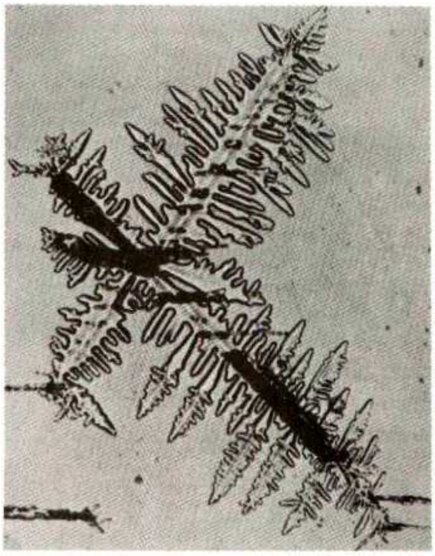
The best piece of advice O'Connor and Woodford give is, "Begin at the end. Write down your conclusions as clearly, precisely and economically as you can, and relate them to the question or hypothesis you have been examining". Furthermore, "A scientific paper should not be the history of an enquiry, but its outcome". And "Never ... submit for publication a paper that has already been published or accepted for publication elsewhere".

In our writing we are told to avoid dangling participles and infinitives, to use active rather than passive voice, and verbs rather than abstract nouns. We should write, "we believe" rather than "it is thought" when referring to our own ideas: this type of circumlocution has been called the "passive of modesty". And it is misleading, because readers do not know whether the opinion belongs only to the authors, or is generally held.

I thoroughly approve of this book. It is packed with good advice, and written in an enviably lucid and readable style. Every library should have a copy. And every scientist, from Professor to student, whatever his nationality, should read it. The best compliment I can pay the book is that I will follow all its recommendations when I write my next paper.

J. Walker

Ice book



Growth of ice from liquid phase

Ice Physics. By Peter V. Hobbs. Pp. xvii+837. (Clarendon: Oxford; Oxford University: London, February 1975.) £29.

IN recent years there has been a great advance in our understanding of the physical processes going on in ice. Although ice has been studied for many decades, there were many aspects of its behaviour that were not understood—in particular, why it has the structure that it does, what the high pressure phases of ice are, and why ice has the electrical and mechanical properties that it does. Not all of these problems have been completely solved, but the great advances made are worth bringing together so that workers in the field can assess the current state of knowledge and can see how work in the different areas may shed light on each other. In this book Hobbs has tried to do this, and the result is a very large book which surveys the whole field, including virtually all the important work and summarising our present state of knowledge.

This book will therefore become an essential reference for anyone working seriously on the physics of ice. It has no competitor for this place. The only other book in this area, N. H. Fletcher, *The Chemical Physics of Ice* (Cambridge, 1970), is a much shorter book with a different purpose. Fletcher introduces ice physics as an example of chemical physics. He selects from ice physics certain areas of interest, and shows how we have learnt to understand them. It is a book suitable for a course, or as an introduction to some important areas of ice physics. Hobbs's book is much more inclusive. It would not be suitable for a course, although certain individual chapters might. It is in the tradition of the monograph summarising knowledge, and it seems to have achieved this to a remarkable degree.

The first of ten chapters deals with the solid phases of the water substance—bonding of water molecules, structure of ice Ih and hydrogen disorder, other phases of ice and amorphous ice and clathrate hydrates. The second chapter concerns electrical properties, including the theory of electrical point defects in ice developed to explain them. The third chapter considers optical properties including luminescence, infrared and Raman spectra and some of the practical consequences. The fourth chapter is devoted to mechanical properties—elastic, plastic and fracture—including the difference between single crystals and polycrystalline ice. The fifth chapter deals with thermal properties and diffusion, including the problem of the heat capacity and zero-point entropy of ice and the diffusion of vacancies in ice. A chapter on surface properties leads on to a chapter on nucleation of ice and two others on growth of ice from the vapour phase and from the liquid phase. The final chapter is on ice in the atmosphere.

The book is remarkably up to date, and even has an appendix which includes results of the International Symposium on the Physics and Chemistry of Ice (Ottawa, August 1972), the results of which were only published about a year before the present book.

The bibliography is arranged so that it can be used as an author index, in that after each reference (which is given in full with the title of each cited paper) the page numbers on which it is cited are given. The conventional subject index and an index to tabulated and graphic experimental data add to the usefulness of the book. It is strongly recommended to anyone who has reason to enquire what we now know about any of the physical properties of ice.

John W. Glen

Scientific basis for practical conservation

Conservation in Practice. Edited by A. Warren and F. B. Goldsmith. Pp. xix+512. (Wiley: London and New York.) Cloth £9.50; paper £4.50.

AFTER fifteen highly successful years, the University College, London, MSc course in conservation is still alive and well, and residing in Bloomsbury, a fact which is demonstrated by the publication of this collection of essays written by many of those who have been involved in the development and teaching of this course. The term 'conservation' has taken upon itself a considerable breadth of meaning in recent years and this is reflected in the wide subject coverage of the thirty chapters in this book. These range over the control of weather, coastlines and floods, the preservation of genes, habitat management (including a number of case histories, for example, heathlands, woodlands and moorlands), pesticides and derelict land reclamation. There are accounts of historical approaches to subjects such as soil erosion, and experimental approaches to problems such as human trampling. And, of course, there are the inevitable accounts of the politics and administration of conservation.

Books could be written about any one of these subjects; indeed books have been written about most of these subjects. It is inevitable, therefore, that many of these short (average 15 pages) essays should be superficial. There are occasions when simplification is taken to the point of inaccuracy, but this is fortunately rare. Perhaps the most successful articles are those which do not attempt to summarise their entire subject, but concentrate on well-documented examples from which general principles may be drawn. For this reason I found Hollis on river management, Norman Moore on pesticides and Vita-Finzi on soil erosion, particularly valuable. It is understandable that difficulties will arise when trying to write short articles on broad subjects aimed at "the layman as well as students, planners, and so on."

One might also question the use of the phrase *in Practice* in the title. Max Nicholson states proudly in the Foreword that the book "was written almost entirely by administrators, planners and official and university scientists without a park superintendent, a forester or a land manager among them". One wonders how this can begin to be a manual of practical conservation; in fairness, it is not. Rather it seeks to emphasise the necessity for a scientific basis for practical conservation.

On the political and administrative side, Nicholson's Foreword must rank as the most entertaining and frank contribution. He decries the recent splitting of the old Nature Conservancy and one cannot but sympathise with his views. One wonders, however, whether it might result in a greater research effort into the practical issues of conservation rather than theoretical ecology. Admittedly, it is difficult to separate the two—only Whitehall seems capable.

Overall, the book's greatest merit is that it draws into one volume contributions from such a variety of disciplines. Any student who picks up this book will find some fresh ideas and information from a field outside his immediate training and, in doing so, he will broaden his concept of conservation.

P. D. Moore

Bumper book on cognition

Exploration in Cognition. (A Series of Books in Psychology.) By Donald A. Norman and David E. Rumelhart. Pp. xvi+430. (Freeman: San Francisco, 1975.) $13.50.

THIS is a sort of academic version of a *Bumper Holiday Fun Book*, recounting all the latest adventures of Professor Norman and his jolly chums by the sea in sunny San Diego. The chums are called the LNR Research Group and they are accompanied on their explorations by their pet computing system, MEMOD.

The book begins with a general discussion of the group's approach to the study of language and cognition, leading to a comparison of the mind with an "active structural network". The important characteristic of an active structural network is that it stores factual information—John is 6ft tall—in the same way that it stores knowledge about how to manipulate that information: how to find out whether John is taller than Bill. It is at the same time a table of facts, and a flowchart for a program that can use the facts in the table to make further deductions. In computer programming terms, it erases the distinction between programs and data.

The group uses the terminology of active structural networks to formulate theories about the strategies we use to carry out various mental tasks, such as recognising faces and playing board games. They also write computer programs to simulate networks, and compare the performance of these programs with that of human subjects. MEMOD, whose name stands for "memory model", is specially designed to simulate active structural networks, and the book gives a clear discussion, at layman's level, of how it works.

In the last half of the book, various members of the group describe their own research projects. Palmer's discussion of ways in which knowledge of the world can guide visual perception, and Eisenstadt and Kareev's work on how people analyse board positions in the games of Go and Go Moku are especially interesting.

The main fault of the book as a whole is a tendancy to let theoretical discussions trail off inconclusively with remarks to the effect that more work needs to be carried out in the area. This seems to be connected with an unwillingness to set up any criteria by which we might judge the adequacy of the group's whole approach, and in particular the use of active structural networks. They are not bothered if a particular theory formulated in terms of these networks doesn't work very well, because they can always formulate another one instead.

Perhaps they are right not to worry. It is clear that there is some interesting work being done in San Diego, and this book conveys the feeling that they are having a good time doing it.

S. D. Isard

Pictorial Key to Genera of Plant-Parasitic Nematodes

FOURTH EDITION, REVISED

W. F. Mai and H. H. Lyon

The only work of its kind, this fully illustrated, easy-to-use manual will be indispensable to students and others who wish to identify nematodes. The descriptive key, full-page photographs, generic descriptions, and characteristics of genera will aid users in learning to differentiate plant-parasitic nematodes from other types that also occur in soil and plant tissue, and to identify them as to genus. Also included are 1500 citations of publications on the taxonomy of the 66 genera and on the general taxonomy of nematodes, particularly plant-parasitic nematodes. 224 pages, £6.35 (paper).

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Circle No. 14 on Reader Enquiry Form.

Bumblebees . . .

Bumblebees. By D. V. Alford. Pp. xii+352+48 plates. (Davis-Poynter: London, June 1975.) £25.

Photo: Stephen Dalton/Natural History Photo Agency

BUMBLEBEES hold a particular fascination for the entomologist because of their social organisation and because they are important pollinators of the native flora and many of our crops. Consequently, their biology has been studied in depth and several monographs have been devoted to them, to which Dr Alford's comprehensive and up-to-date account is a welcome addition.

The layout of the book is good. There is a brief introduction outlining bumblebee anatomy and physiology followed by the three main parts of the book. Part I includes chapters on various biological aspects of colony life, including hibernation, colony initiation and development. The author has confined himself largely to nest biology and says relatively little of their foraging behaviour and ecological importance as pollinators. The author's particular interest is reflected in the large section devoted to the parasites and predators of bumblebees. Part II deals primarily with the British species; each is described with information on their classification, habits and distribution. In Part III methods of collecting individual bumblebees and of locating, transferring and housing their nests for observation are outlined. The appendix presents 26 distribution maps of the British species of bumblebees as prepared by the Biological Records Centre for the Bumblebee Distribution Maps Scheme, for which Dr Alford is collating data sent in by members of the Bee Research Association. A discussion of how the distribution of different species is changing would have been interesting but perhaps little is known. Lists of all the plant and animal species mentioned in the text are given and there are comprehensive author, plant and animal indices and a bibliography of over 500 references to original papers.

The book is copiously illustrated with 26 colour plates, 63 black and white plates and over 200 line drawings. The photographs are, however, disappointing. In his determination to present photographs of all the British species, the author has included many of poor quality. The line drawings are clearly drawn and enhance the text, although many of them would appear more attractive if they had been reduced in size.

The volume is expensive. It will certainly be too highly priced for many individual pockets but should be a useful addition to specialist libraries.

Ingrid H. Williams

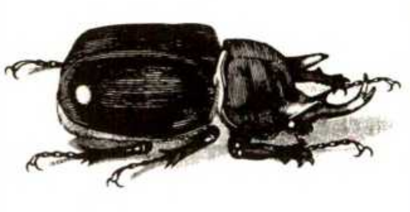
. . . and beetles

The Life of Beetles. Glyn Evans. Pp. 232. (Allen and Unwin: London, June 1975.) £6.90.

As one of my mentors used to say, "God made too many beetles". Until now there has been no book on the general biology of beetles in English, and such a book is therefore bound to fill a need. Dr Evans has not, however, written an impressive book. Much more information about them can be conveyed in 200 pages even without the use of "specialist jargon", the absence of which sometimes does duty here for a lack of knowledge. The book lacks balance. For instance, more than a sixth of the text is a chapter on food and feeding habits. Here we find nothing about the vitamin, amino acid, and other nutritional requirements of beetles nor about the role of gut and intracellular symbionts or how beetles utilise such materials as cellulose and keratin. Some of the illustrations are good, but a few are exceptionally poor (for example, Fig. 3). The references are at the end of the book, but for some reason they are cited separately for each chapter. One consequence of this is that in a meagre bibliography no less than 15 titles occur two or even three times in seven pages. In a section "Further Reading" no less than nine titles from the bibliography are repeated, sometimes twice.

Lucanus cervus (*stag beetle*), *from a drawing made in 1805*

Mary Evans Picture Library



Scarabaeus acteon (*scarab beetle*)

Mary Evans Picture Library

In the introduction and elsewhere the role of the hard and rigid body-wall cuticle in the success of beetles is heavily stressed, but most beetles are larvae and most larval beetles have a soft and flexible body-wall. Taenidial thickenings are not lost in the finer branches of the tracheae (p. 51): they are present even to the ends of the tracheoles. The visible spectrum of insects does not end at blue-green (p. 43) but extends from far below 300 to about 750nm in a few. No other group of animals has so wide a spectrum. The larvae of the Dryopidae are said to be aquatic (p. 29), but there is no authentic instance of an aquatic larva in this family. In the Hydrophilidae silk is not produced by the Malpighian tubes (p. 68) but is secreted by colleterial glands. He suggests (p. 68) that the funnel of the cocoon of *Hydrophilus* is a "tail-piece" of no functional significance, but it was long ago shown that it enables the embryos and young larvae to use atmospheric oxygen while the cocoon is covered by water.

There is a very real need for a book on the biology of beetles that combines wide personal knowledge of the animals with extensive reading. The desire to write a book on beetles is not of itself enough.

H. E. Hinton

Snails, slugs . . .

Pulmonates. Volume 1: *Functional Anatomy and Physiology.* By Vera Fretter and J. Peake. Pp. xxix+417. (Academic: London and New York, April 1975.) £13.20; $35.

Mary Evans Picture Library

Snails meeting on a vine branch, from an etching by M. Giacomelli

FROM the title, I wondered momentarily whether Fretter and Peake have produced a pulmonate sequel to the superbly integrated and illustrated Ray Society volume, *British Prosobranch Molluscs* (Fretter and Graham, 1962). Time is perhaps not ripe for such a volume. Considering the present state of our knowledge of the pulmonates, a challenging and daunting task has been undertaken in producing the present work.

In her introduction Fretter compares some aspects of the functional anatomy of prosobranch, opisthobranch and pulmonate gastropods and briefly considers their possible relationships and the origin of pulmonates from prosobranch stocks. She draws attention to the frequent differences in terminology used by prosobranch and pulmonate workers.

This volume brings together eight reviews covering the fields of locomotion, respiration, alimentary canal, water relationships, nervous system, endocrinology, reproduction and development. These reviews have been contributed independently by different authors and thus differ in form, in the selection of their content and in the period of time over which the author ranges; and several concentrate on research of the last ten years, that is, subsequent to the writing of Wilbur and Yonge's *Physiology of Mollusca.* Often, more and clearer illustrations would have been welcome, but all authors provide extensive reference lists—surprisingly without titles.

A valuable feature of this book is the systematic index. Within the context of the book, it pinpoints those species for which substantial data now exists, whilst indirectly indicating the limited number of pulmonate species on which some physiological work has been carried out. Sadly we must await volume 11, which deals with "the ecology of the group, some aspects of its economic importance, systematics and evolution", to relate the species utilised and the results they yield to their ecological niche and their systematic position. For convenience, a summary of the classification of pulmonates should have been included in this volume.

The authors are frank when presenting conflicting results—for example, page 291 ". . . contradictions can be expected by considering that not only different species were used, but that also the experimental conditions were not uniform . . .". Comments on problems, and the vast amount of work still necessary to unravel the complexities of functional anatomy and physiology among the pulmonates certainly make this book a stimulant to further research. As a record of what has been achieved in many areas and a pointer to what can be done, this book should take its place on the shelves of research departments.

Joyce E. Rigby

. . . and worms

The Locomotion of Soft-Bodied Animals By E. R. Trueman. Pp. viii+200. (Edward Arnold: London, June 1975.) Boards £8.50; Paper £4.25.

LIFE became mobile when animals evolved. Their movement, however, brings to mind the flight of birds or insects, the speed of horses, the mastery of swimming by fish and whales. But such creatures with highly efficient muscles working in antagonism to rigid internal or external skeletons are final productions of evolution. Humbler animals must be contemplated if we are to appreciate how mobility evolved and is still practised by major sections of the animal kingdom searching for food or seeking protection.

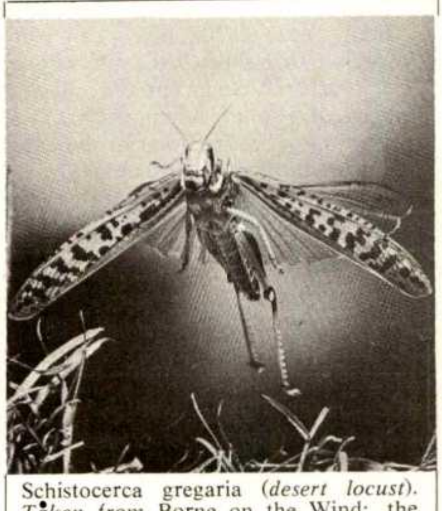
This book deals with movement in coelenterates, in worms from turbellarians to polychaetes, with some concern for echinoderms and much for molluscs. In evolution of the Metazoa, the solid flattened body suitable for progression with cilia over hard surfaces gave place to a rounded form with fluid-filled cavities —coelom, haemocoel or pseudocoel— before soft substrates could be penetrated. An infauna exploring new sources of food and avoiding the attention of evolving carnivores came to supplement the original epifauna.

A muscular system acting in antagonism to a fluid skeleton provided a capacity to burrow—most simply exhibited by anemones such as *Peachia*— and more elaborately in various worms, and to the highest degree in the segmented polychaetes. Protrusion of the muscular foot with extensive haemocoelic cavities has enabled molluscs, primarily bivalves but also scaphopods and various gastropods, most efficiently to progress through soft substrates.

The process of movement in surface-crawling worms and snails has long been studied but the interpretation of movement within the substrate had to await elaboration of suitable measuring techniques, notably the Statham pressure transducer. So equipped, Professor E. R. Trueman and co-workers have revealed the sequence of events in the subsurface movements of representatives of all types of soft-bodied burrowers. Certainly to this reviewer, their success seems to be greatest where knowledge was least, in the interpretation of the digging cycle in bivalves leading to boring into rock or timber. The supreme achievement of movement in soft-bodied animals is, however, the jet propulsion of the molluscan squids, involving an elaborate system of antagonistic muscles under precise nervous control.

This concise, well written and illustrated book associates and interprets a range of experimental data to provide functional interpretation of much invertebrate structure. No one can read it without gaining a better understanding of the animal kingdom, surely the prime aim of the study of zoology.

C. M. Yonge



Schistocerca gregaria *(desert locust). Taken from* Borne on the Wind: the Extraordinary World of Insects in Flight. *By Stephen Dalton. Pp. 154, Hogarth: London, October 1975.) £5.00.*

Announcements

Award

The Belgian Academy of Medicine has awarded the Pfizer Prize 1972–1973 to **Professor Jacques E. Dumont** of the Institute of Interdisciplinary Research, School of Medicine, University of Brussels.

Appointments

Dr Arturo Gomez-Pompa has been elected chairman of the International Co-ordinating Council of UNESCO's Man and the Biosphere Programme.

Dr Frank Read has been made a Professor in the Department of Physics at the University of Manchester.

Professor Sela has been inaugurated as president of the Weizman Institute.

International meetings

January 28–29, **The northern great barrier reef,** London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

February 2–6, **Nuclear techniques in animal production and health as related to the soil–plant system,** Vienna (H. D. Hamel, Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, Kärntner Ring 11, P.O. Box 590, A–1011 Vienna, Austria).

February 4, **Replacement of biological methods by chemical and physical methods,** London (Chemical Society, Burlington House, London W1V 0BN, UK).

February 5–7, **Applied clinical immunology,** Houston (The Office of the Director, The University of Texas Health Service Centre at Houston, Division of Continuing Education, P.O. Box 20367, Houston, Texas 77025, USA).

February 11, **Techniques for the determination of traces of volatile compounds,** London (Chemical Society, Burlington House, London W1V OBN, UK).

February 18, **Analysis of pesticides,** Chesterford Park (Chemical Society, Burlington House, London W1V 0BN, UK).

February 18, **Adhesion of thin films,** London (The meetings officer, Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

February 18–19, **Contraceptives of the future,** London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

February 26–27, **Methods and applications of ranging to artificial satellites and the moon,** London (The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG, UK).

Reports and publications

Mammals of the Wankie National Park, Rhodesia. By V. J. Wilson (Museum Memoir, No. 5). Pp. 147. (Salisbury: The Trustees of the National Museums and Monuments of Rhodesia, 1975.) [1010

Congress Against the President. Edited by Harvey C. Mansfield, Sr. Pp. 200. (New York: The Academy of Political Science, 1975.) [1010

Smithsonian Contributions to Zoology, No. 202: Cyclopoid Copepods (Nanaspididae and Sabelliphilidae) Associated with Holothurians in New Caledonia. By Arthur G. Humes. Pp. iii + 41. (Washington, DC: Smithsonian Institute Press, 1975. For sale by US Government Printing Office.) [1010

Publications of the United States Naval Observatory, Second Series. Vol. XXIV, Part 1: Third Catalog of Trigonometric Parallaxes of Faint-Stars. By R. S. Harrington, *et al*. Pp. 30. (Washington, DC: US Government Printing Office, 1975.) [1010

Smithsonian Contributions to the Earth Sciences. No. 13. Distinctive Properties or Turbiditic and Hemipelagic Mud Layers in the Algéro-Balearic Basin, Western Mediterranean Sea. By Nicolaas A. Rupke and Daniel Jean Stanley. Pp. iii + 40. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 95 cents. [1310

United States Department of the Interior: Geological Survey. Bulletin 1395-H. Geologic Setting of the Glacier Peak and Mazama Ash-Bed Markers in West-Central Montana. (Contributions to Stratigraphy.) By R. W. Lemke, M. R. Mudge, Ray E. Wilcox and H. A. Powers. Pp. iii + 31. Professional Paper 877: The Black Hills-Rapid City Flood of June 9–10, 1972: A Description of the Storm and Flood. By Francis K. Schwarz, Lawrence A. Hughes, E. Marshall Hansen, M. S. Petersen and Donovan B. Kelly. Pp. vi + 47. (Washington, DC: Government Printing Office, 1975.) [1310

Smithsonian Contributions to Zoology. No. 206: The Echinoids of Carrie Bow Cay, Belize. By Porter M. Kier. Pp. iii + 45. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.) [1710

Theories of Nature and Physics. By Herbert A. Bosch. Pp. 40. (Box 12, Little Falk, Minnesota: Herbert A. Bosch, 1975.) [1710

United States Department of the Interior: Geological Survey. Water-Supply Paper 2123: Surface Water Supply of the United States, 1966–70. Part 8: Western Gulf of Mexico Basins. Vol. 2: Basins from Lavaca River to Rio Grande. Pp. ix + 861. $5.70. Water-Supply Paper 2134: Surface Water Supply of the United States, 1966–70. Part 13: Snake River Basin. Pp. ix + 821. $5.50. Wzter-Supply Paper 2155: Quality of Surface Waters of the United States, 1970. Part 6: Missouri River Basin. Pp. xi + 554. (Washington, DC: Government Printing Office, 1974 and 1975.) [1710

Answers to Christmas quiz

1. (*a*) Not necessarily (see *Nature*, **256**, 206; 1975).

(*b*) Nägeli the botanist and other naturalists failed to recognise their importance; Mendel became involved with administration on his appointment as abbot of his monastery, and as his girth expanded Mendel found it increasingly difficult to reach his pea plants (especially the short breed).

2. Norbert Wiener entered college at the age of 11 and gained his PhD at 18. The others were retarded to the extent of only starting college at 14/15 and not obtaining their doctorates until 21/22.

3. Both had to overcome a severe physical handicap. Sumner had his left arm amputated as a teenager. Cornforth has been completely deaf from birth.

4. The female deep sea angler fish can be 500,000 times heavier than the male.

5. Notorious, directed by Alfred Hitchcock. Professor R. A. Millikan told him that the whole idea of an atomic bomb was ridiculous, but the FBI still kept Hitchcock under surveillance for 3 months.

6. (*a*) *Twenty Thousand Leagues Under the Sea* by Jules Verne;

(*b*) *The War in the Air* by H. G. Wells;

(*c*) *The Unparalleled Invasion* by Jack London;

(*d*) *The Space Merchants* by Frederick Pohl and Cyril Kornbluth.

7. Letters and postcards between Albert Einstein and Willem de Sitter were found at Princeton and Leiden. They discussed the nature of the Universe.

8. In January this year Dr J. D. Isaacs and colleagues suggested that cars driven constantly on one side of the road can create atmospheric vorticity, leading to the formation of tornadoes (see *Nature*, **253**, 254; 1975).

9. When deprived of vitamin B_{12}, some bats develop symptoms of demyelination. They may provide a useful system for research (see *Nature*, **254**, 148; 1975).

10. Amphetamine, synthesised in 1927, was first used clinically in 1935. It produces a state of well being, decreases appetite and increases physical activity.

11. There is an excess of persons born in the first 3 or 4 months of the year in samples of schizophrenic patients. This is called the season-of-birth effect.

12. In 1665 *Philosophical Transactions* was founded by Henry Oldenburg, Secretary of the Royal Society.

13. David Davies, editor of *Nature*, seen through the eyes of daughter Rebecca (see *Nature*, **254**, April 3; 1975, front cover).

14. Charles Wheatstone (see *Nature*, **256**, 532; 1975).

15. Members of the National Astronomy and Ionosphere Center at Cornell University in a message beamed to globular cluster M13, 24,000 light years away. *Nature*, failing to appreciate the subtleties optimistically aimed at our potential cosmic correspondents, printed the pictorial message the wrong way up. (see *Nature*, **253**, 230; 1975).

16. Most people working on the structure of chromatin (see *Nature*, **257**, 177; 1975).

17. (*a*) Miguel de Unamuno, *The Tragic Sense of Life*, *page* 90;

(*b*) O. W. Holmes, *Medical Essays*, page 211;

(*c*) Plato, *Theoetetus*, section 1.82;

(*d*) Adam Smith, *The Wealth of Nations*, book 5, part 3, section 3.

nature

December 18, 1975

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Every company a teaching company

BRITISH industry is in a pretty bad way. Its share of the world market and of the domestic market has declined steadily for many years, and there is no feeling that anything is coming along to save it from even further decline. The per capita output of British industry is only half that of industry in France, Germany, Japan and the United States; every other statistic tells the same gloomy story.

There are a hundred and one remedies offered, many of which are directed at increasing investment or improving labour relations, and in the past few years the Science Research Council (SRC) has itself looked increasingly at the problem of the intellectual input into industry. For it is undeniable that British industry has consistently failed to attract enough bright graduates and PhDs, and has not given enough scope and status to those it has employed. In efforts to stimulate the university–industry links, which seem to come much more naturally in other countries, the SRC and associated government departments have launched a variety of ideas: 'Bosworth' courses, Cooperative Awards in Science and Engineering (the CASE scheme), Total Technology, joint SRC/Social Science Research Council postgraduate training, academic–industrial collaboration schemes, a revamped PhD . . . and now, the teaching company.

In a report just issued (*The Teaching Company*, available from the SRC, free) a joint working party of the SRC and the Department of Industry propose that two or three companies should be added annually to a list of those in which postgraduate students could spend, say, two years working towards a higher degree with a mixture of formal courses and on-the-job projects planned to improve the company's manufacturing operations. Perhaps half a dozen students would be involved in each company; already plans are being made to start experimental operations in four companies. The students will not be doing research in the strict sense—they will be learning about manufacturing technology from practical examples while keeping one foot in a university. Proponents of the teaching company idea freely admit that it is largely modelled on the teaching hospital concept.

The hope is that a corps of well equipped graduates will emerge from the scheme, with experience of running an industrial show and ability to talk to universities about industrial needs in manufacturing technology. These graduates would then start, as one member of the working party put it, to create oases within the present industrial desert. Those who have passed through a teaching factory should be obvious candidates for posts as production directors, or even managing directors, of companies before too long.

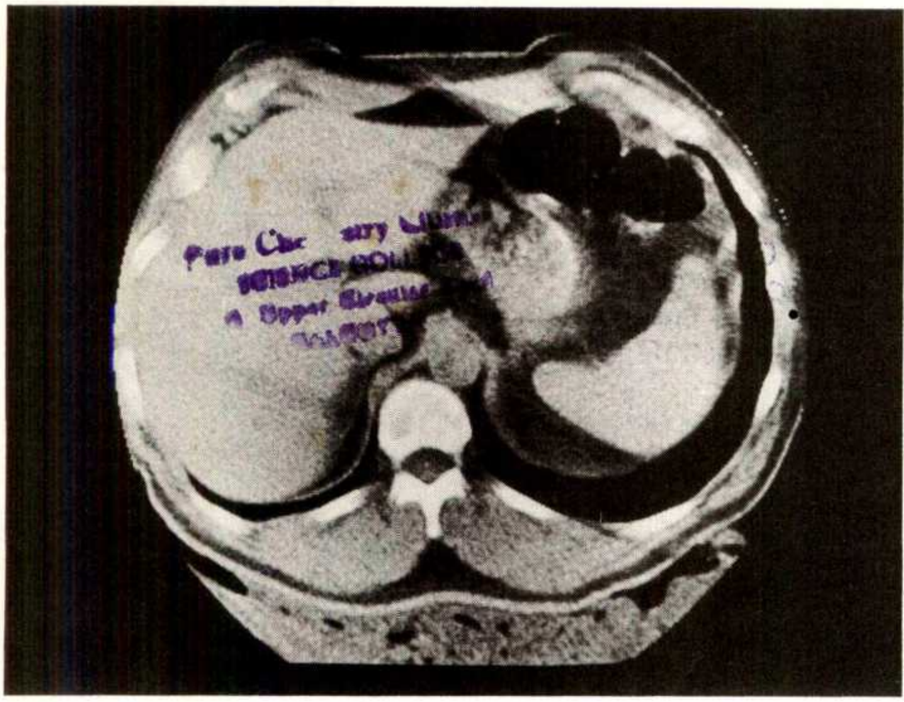
One's first reaction may well be surprise that this sort of thing is not happening already. Surely, in view of all the plans with which SRC have regaled us over the past few years, graduates ought to be moving more freely between university and industry. The problem seems to be that manufacturing technology has always been lowly rated both by hide-bound industry dubious of the need to hire graduates and by universities which find that the subject does not sit too easily among other more academic pursuits. But is the teaching company the answer? There are some serious objections to be considered, and most of these are a matter of scale.

If a mere handful of companies are to be so designated, and these participate voluntarily, indeed willingly, they are already set apart as remarkably enlightened organisations which, quite rightly, probably stand to profit substantially from their involvement.

Embryo manufacturing engineers also need to know about the just-average companies and even the appalling ones. Otherwise there is a danger that on emerging from the programme they will recoil in horror at the general industrial landscape and return to work at the teaching company! There must also be some doubt that even the best run company can provide a continuous flow of good projects to which students can contribute significantly in two years without feeling that the whole thing is being trumped up and is irrelevant to the company's real operations. Finally, many recent initiatives to bring universities and industry closer together have only met with real enthusiasm in new universities; the older ones have viewed innovative programmes as not their concern. Unless the idea of the teaching company can be as acceptable in Oxford as it is in Salford, the proposal will have a diminished impact.

Industry's problems in getting and using graduates are, at root, social ones. Class distinctions, (even within the professions, let alone between workers and management), attitudes of school-children to engineering, the prestige of pure science, anti-intellectualism, pay scales—these all contribute to the bind in which industry finds itself. A handful of teaching companies is not enough. Should not every company be prepared to help train graduates?

●



Scan of abdomen showing liver, pancreas and spleen (A'court photographs)

Harnessing electronics to medicine

As world resources are turned increasingly towards ameliorating environmental problems and improving the health of the population, the growth of electronic technology in medicine is well under way. But one of the crucial factors that will determine the advance is finance. Bernard Watson reports on a trend now represented most popularly by the EMI scanner.

ANYONE who has found himself in a hospital at various times since the Second World War could not have failed to notice the increasing sophistication of the equipment used to diagnose ailments and monitor the progress back to health. Modern technology and medicine now work hand in hand, and continue to open up new vistas.

The development of machines for the automatic chemical analysis of blood and new radio-immuno assay techniques for hormones has contributed to a rapid increase in laboratory diagnostic techniques. Ion sensing electrodes will soon be available which will bring back to the bedside the measurements hitherto made in the laboratory; indwelling electrodes, being miniaturised versions of those used for *in vitro* determinations, will be used for continuous monitoring, while semiconductor probes, such as ion sensing field effect transistors, may also become available.

Laboratory measurements like these supplement the measurements made by direct attachment to the patient. Cardioscopes and arrhythmia detectors, which may include computer surveillance of patients, are to be found in the intensive care and coronary care wards, as are automatic respirators. Indeed, the whole array of life support systems has reached a high degree of complexity.

Therapeutic advances have also been made. The electronic pacemaker is now accepted and even implanted in the aged patient. The artificial kidney has advanced from the early Keel parallel plate dialyser, using cellophane as a membrane, to the disposable capillary kidney, though the cost remains a major disadvantage. This could give way to the wearable kidney with absorbents, or to the development of the storage of donor organs to such a degree of sophistication that matching can take place over months instead of more than 48 hours.

A prosthesis for the patient who has lost a limb will soon include electronic feedback from muscle groups that are still able to signal the brain's commands, provided implantation of the device is accepted. Electronic aids for the blind will also improve. And if the electrical stimulation of the visual cortex to produce significant emanation of light (or "phosphenes") can be harnessed, together with a reduction in size of the solid state camera, the 'artificial eye' will be realised. A prosthesis for the deaf based on the production of "audenes", analogous to the "phosphenes", may be within reach as well.

Doppler ultrasonic blood flow measurement is now accepted as a useful technique for the vascular surgeon as well as the obstetrician, who uses it to record the foetal heart. Here, the ultrasonic energy is continuously transmitted, unlike the pulsed ultrasound used in echo scanning techniques. The red cell acts as an effective scatterer, and on the assumption that the velocity of the red cell and of the blood as a whole are correlated, it provides a moving target to follow in the vascular system. These meters at present measure blood velocity, but it should be possible to produce quantitative flow meters once the correct method of frequency analysis of the scattered ultrasound is discovered and techniques are developed for measuring the diameter of the artery.

The application of ultrasonic scanning has introduced a new dimension. Small piezoelectric crystals producing 1.5 or 5 MHz ultrasound are coupled by means of oil or water to the skin. The crystals transmit and receive echoes from interfaces separating media of differing acoustic impedance. The third ventricle in the brain is just such a surface, so its displacement, which indicates intracranial pathology, can be measured with moderate ease.

The use of 'A scan' techniques in cardiology for recording the movement of the mitral valve enables the calcification of its leaflets to be assessed by non-invasive means. The separation of the myocardium and the epicardial sac due to infection can also be diagnosed and allows progressive changes to be assessed. The size of the heart and the dimensions of the left ventricle can also be measured. And switched crystal arrays will enable dynamic studies to be made, enabling the volume changes of the ventricle throughout the cardiac cycle to be measured using the techniques developed for angiocardiography. At present, contrast media must be injected and rapid multi-shot X-ray techniques applied.

'B scan' ultrasound has made a significant contribution in gynaecology and obstetrics. Foetal life and growth can now be followed throughout pregnancy, and the placenta can be accurately located. Liver and kidney scans are also useful for the detecting of abnormal pathology.

The recent advance in thermography is also relevant, since the speed and resolution of the latest instruments permits the easy detection of perforating veins and the observation of peripheral vascular changes without the disturbance to the circulation caused by injecting contrast media or radioactive isotopes. It is also of use for detecting deep vein thrombosis. Although mammography using xeroradiography may be used for confirmation, the X-ray dose is still higher than that preferred for routine screening purposes, and thermography will be very useful for six-monthly checks.

These are but a few of the electronic devices which are available to the clinician. The most important advances, however, have been in diagnostic imaging. Previously the radiologist struggled for better definition of pathological and functional change in his patient using conventional X-rays with source, patient and plate in simple configurations. Now, the position of the patient and tube and the tube voltage can be more easily manipulated for better definition.

But the outstanding advance in this field has been the X-ray scanner developed by Geoffrey Hounsfield for EMI. Before the advent of this technique, the clinician had to rely on a barrage of techniques to detect intracranial pathology. The techniques depended on the preferential uptake of a radioactive isotope by the tumour, or the experienced eye of the neurophysiologist looking for changes in the EEG. Final confirmation involved an angiogram or an air encephalogram with their attendant risks.

Hounsfield's approach involves the measurement of the absorption of the soft tissue displayed, but its sophistication is that it gives a definition not seen before in radiology. The initial EMI scanner, moreover, which could only be used for the head, has been followed by a body scanner. The disadvantages of standard tomographic procedure are the indistinct picture of the layer under scrutiny and the superimposition of the blurred images of neighbouring layers.

In the body scanner, the patient is X-rayed as a series of 13 mm thick tomographic sections. Each section is examined during each 20-second scanning sequence. The X-ray source and the detector array are mounted on a scanning frame contained in a gantry. A further detector records the intensity of the primary beam. The section is scanned linearly across the patient and the transmission intensity recorded, producing more than 18,000 readings during the scan. At the end of the scan the frame is moved 10° and a further linear scan commences. The sequence is then repeated through a total of 180°.

The readings of intensity are digitised and analysed by a minicomputer to produce the absorption values at 80,000 points within each section. The magnetic store can store eight 13-mm sections. The information for each slice is recorded in the form of a matrix of square picture elements contained within a circle of 320 picture points. A typical element represents an area in the slice of 1.25×1.25 mm when the machine is set up for a 400-mm body diameter, and 0.75×0.75 mm at the smallest body diameter setting of 240 mm. The absorption values are expressed in units from -500 to $+500$, with water at 0 on the scale.

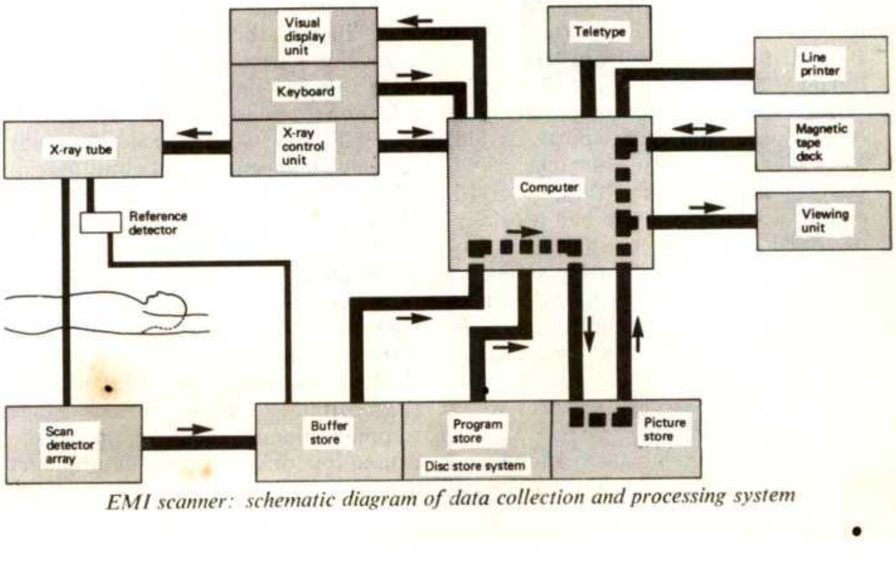
As a wide range of absorption values are recorded during the scanning procedure, a control allows a specific range of values to be displayed. The values are permanently recorded, so any range can be displayed on the television monitor. Bulk storage of patient data is readily available using magnetic tape. For the first time accurate measurements of absorption values to $\pm 0.25\%$ are possible with adequate resolution for the analysis of pathological changes in the organ under examination. Clinical assessment of this technique is awaited, but early pictures demonstrate the detail that can be seen even by the inexperienced reader of radiographers.

Therapeutic applications must follow this development since the definition in the image gives positional information not previously available. The instruments for non-invasive investigation of most patients is now possible without trauma or risk of death, although the inherent danger of exposure to radiation remains. Better therapeutic techniques, and improvements in preventive medicine, are being sought, but therapeutic advances are not yet keeping pace with diagnostic skills.

If technology can produce advances in therapy and preventive medicine as well as contribute to improved social conditions, the recent advances in medicine will be fully realised and the new diagnostic techniques will produce more than just accurate data for analysing failures. Much, however, depends on the crucial factor of finance. In the UK, research and development in medical electronics is considerable but scattered over many hospitals as well as industrial and government research laboratories with little co-ordination. The Department of Health and Social Security (DHSS) has tried over the past 10 years to encourage specific developments where it has been able to define a need. Pacemakers with ever-greater reliability provide one example and work on these contributed to the development of the miniature nuclear battery for longer-life pacemakers. Companies will generally look for a promising home market before they invest since the overseas medical market is difficult to service and brings its own problems in differing safety standards and clinical approaches. In the case of the initial prototype EMI scanner, finance was provided jointly by the DHSS and EMI.

More could be done if resources were available, but the cost of development is escalating. Development facilities are presently lacking in the hospitals where therapeutic development could be done cheaply and effectively because of the close contact with clinical problems. A capital grant alone will not solve the problems, however. Trained personnel such as engineers and technicians are also needed to use the equipment and integrate it successfully into clinical medicine if there is to be more effective diagnosis and treatment. The development funds of the DHSS have increased more than five-fold during the past 10 years, but the exact level of expenditure is difficult to assess because of the diversity of the programme.

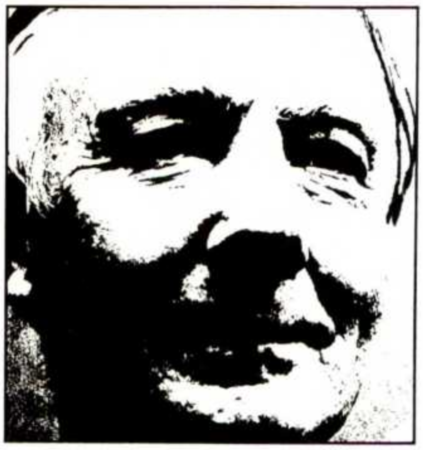
Health expenditure must increase as medical science unravels more of the diversity of disease processes. But the requirement that it be cost effective often causes administrators to wonder whether too much money is devoted to interesting but rare diseases. Animal experimentation might be preferred and it has its place, but it will never surpass carefully collected data from



EMI scanner: schematic diagram of data collection and processing system sick patients. On the other hand, the clinician has little time to undertake applied research. Considerable improvement in medicine would occur if money could be provided to finance multidisciplinary teams on well-planned projects in which industry would collaborate by developing the necessary technology.

But at the moment the need to treat more patients than ever before clashes with increasingly rigid budgetary constraints. As in so many areas, but particularly in research, a world of priorities means that the capacity to find technological solutions might exist but the economic encouragement often does not. □

Attending a Moon conference



KENNETH MELLANBY

I have just returned from attending the Fourth International Conference on the Unity of the Sciences, held at the Waldorf-Astoria Hotel in New York from November 27–30. The subject of the conference was "The Centrality of Science and Absolute Values", and it was attended by some 350 of what the programme describes as "the world's most eminent scholars and scientists" from 60 different countries. Readers of *Nature* will remember the editorial, "Bringing men to the Moon", published on October 25 1974, just before the Third Conference was held in London last November. The editorial questioned the status of the International Cultural Foundation, which was sponsoring (and financing) the conference.

The Foundation was started by the Reverend Sun Myung Moon, who is also the founder of the Unification Church. This church has been severely criticised, mainly for its strong anti-communist views, and for what some consider its undue influence on young converts. The *Nature* article suggested that the participants in the conference might find that they were being used to give respectability to an organisation of which they did not entirely approve. Although the ICF exists as "a non-profit organisation dedicated to promoting academic, scientific, religious and cultural exchange among the countries of the world", critics have suggested that its aims may be less disinterested and more political.

The Fourth Conference included a few representatives from communist-governed states. Invitations had been sent to several people in Soviet Russia, but these were not accepted—an experience not unknown to organisers of the most orthodox scientific gathering. But scientists came from Hungary, Poland and Yugoslavia. In 1974 India was not represented, but this time several Indians attended, as did Egyptians, Lebanese, Israelis, and many others from "third world" countries. As before, we had a galaxy of Nobel Laureates, in physics, chemistry, physiology and other subjects. Several theologians, including Jesuits and other Roman Catholics, as well as members of other sects and different religions, were among the participants. The social sciences were well represented.

Last year the advance announcements about the Third Conference listed eminent names of supporters and participants, some of whom later dissociated themselves with the organisation. The opinion was stated (in *Nature* and elsewhere) that this might have been done to try to secure wider support. I believe that the use of any names without permission was due to misunderstanding—I know one Briton who was approached who said he could not attend but "'would help if he could", and then objected when he was listed as a supporter. This year I do not think that anyone was named who was not genuinely willing to help.

Critics of the ICF have queried the selection of those invited, and of the programme of the conference. They have suggested that members may have allowed themselves to be put in a position where they could not freely state their views. Regarding the selection: the invitations came from the ICF, but names were chosen by entirely independent committees of advisers, American and international (I must confess to being one of their number). I do not know of any case in which a name was suggested and the individual was not invited. It is true that the majority of members were middle-aged, and that few strident marxists were included but they certainly included a wide range of opinions and beliefs. Any gaps were caused by refusals, and by the fact that the advisers did not wish to include those, however well intentioned, who would wreck the event. In fact a few uncompromising critics did attend.

There was no censorship of views or opinions whatever. The 120 or so papers, which were previously circulated to the groups which discussed them, were read by the committee chairman, and sometimes authors were asked to shorten their text, but no matters of substance were excluded. As one who chaired a discussion group I can confirm that no one was muzzled. The conference certainly cannot be criticised as not allowing complete freedom of expression to all participants.

The $500,000 question remains to be answered: was the conference worth while? I personally think that the answer is "yes". Many equally expensive international gatherings, even those dealing with important scientific topics, are clearly a waste of time and money. Little new is said or heard—"Professor Blank read his paper" (the same one read so often before). Here we had a gathering of good men and women with good minds who seriously discussed the problems facing the world today, and the ways in which science could and should be involved.

I know of no other gathering where this would have been possible. There was less nonsense talked than might have been expected—in my opening address I echoed a warning in last year's *Nature*, when I said that the present disenchantment of the public with science and scientists was "sometimes generated by scientists themselves—when they appear to speak as experts in fields where they possess no expertise".

We attempted to define the boundaries of the various branches of science, and the relations between the natural and social sciences. We discussed the problem of values, in regard to the family, to religion and to morality. Consideration was given to problems facing universities and research institutions, and to the ethics of the application of modern technology. The position of science in resolving world inequalities and in the creation of any fuure world order was also discussed. We did not solve all the world's outstanding problems, but perhaps we made some progress in the right direction.

international news

Genetic manipulation: recommendations drafted

from Colin Norman, La Jolla

AN ADVISORY committee of the National Institutes of Health (NIH), which met here on December 4 and 5, has drafted a set of strict regulations to control the use of a revolutionary, but possibly hazardous, technique for manipulating genes from living organisms. Its action, which follows several months of controversy and uncertainty, is the latest step in a tortuous process of self-regulation by a segment of the scientific community. Although its recommendations will apply only to research supported by the NIH, they are likely to have broad international implications, because several other countries—including Britain—are also going through the difficult business of trying to draft regulations governing use of the controversial genetic manipulation procedure.

The technique, in short, involves the use of recently discovered enzymes, called restriction enzymes, to rearrange genetic material in combinations which are unlikely to have occurred by natural evolution. For example, it enables genes to be snipped from the DNA of virtually any organism and inserted into a virus or a bacterium in such a way that they will be copied each time the virus or bacterium reproduces (a process known as cloning).

Such direct genetic manipulation has already opened up some exciting areas of research because it offers a relatively simple means of multiplying and studying genes from various organisms, including man. And, more distantly, it may have potential applications in medicine, agriculture and industrial processes, such as correcting genetic deficiency diseases, inserting nitrogen-fixing genes from bacteria into non-leguminous plants so that they would no longer require nitrogen fertilisers, and constructing novel strains of bacteria for such tasks as eating up oil spills.

But the technique also involves some potential hazards, the nature and likelihood of which can only be speculated upon. One worry, for example, is that viruses or bacteria bearing genes transplanted from another organism could conceivably be endowed with unpredictable biological properties such that, in the worst imaginable case, a novel epidemic could be let loose if they were to escape from the laboratory. A particular problem is that unknown bits of DNA may be transplanted along with the genes under investigation, which opens up the remote possibility that, for example, cancer-causing genes could be introduced into a virus or bacterium and cloned. And such fears are heightened by the fact that most of the experiments are carried out on a laboratory strain of the bacterium *E. coli*, a microorganism which is a common inhabitant of the human gut.

Although most of the potential horror scenarios are highly speculative, and there are no experimental data to confirm or deny that they are plausible (see box), few scientists are prepared to state that the technique is entirely safe. Consequently, in July 1974, a committee of the National Academy of Sciences, led by Paul Berg of Stanford University, issued an unprecedented call for a voluntary moratorium to be placed on some uses of the technique, at least until the possible hazards have been evaluated and some regulations have been drawn up. A meeting of some 140 geneticists from various countries was subsequently convened last February at Asilomar, California, to discuss the intricacies of the matter and recommend some guidelines to the scientific community.

Paul Berg: "happy" with draft

The Asilomar conference was faced with a tough dilemma. Although the voluntary moratorium seemed to have been universally respected, there was already some agitation for it to be lifted because it was holding up potentially valuable research; but experience with conventional laboratory safety procedures does not inspire much confidence that viruses or bacteria bearing transplanted genes could be prevented from escaping into the environment, with possibly dire consequences. Many laboratory workers, for example, have suffered infections as a result of research on hazardous pathogens, and the 1972 London smallpox outbreak, which originated in a research laboratory, amply demonstrated how conventional safety procedures can break down.

The Asilomar conference hit on a neat solution to the dilemma, however. It recommended that, in addition to the use of conventional, physical safety precautions, potentially hazardous experiments should only be carried out on viruses or bacteria which have been genetically crippled so that they would be incapable of surviving outside an artificial laboratory environment. It was generally anticipated that such crippled strains could be produced within a matter of weeks, and everybody could soon be safely back at work.

But, unfortunately, some of the optimism turned out to be unfounded, because attempts to develop a crippled strain of *E. coli* have run into numerous snags, and parallel efforts to disable a strain of virus known as bacteriophage lambda, which infects bacteria and which is a possible vehicle for inserting genes into *E. coli* for cloning, also took longer than anticipated. Until such strains are available, the Asilomar guidelines specify that the voluntary moratorium should be maintained for many kinds of experiments. Consequently, most planned experiments have effectively been under an embargo for 18 months.

It is in that difficult situation that the NIH advisory committee has been trying to write acceptable regulations from the Asilomar guidelines. And it has also been working in an atmosphere charged with suspicion and unconfirmed rumours that embargo-breaking experiments are being carried out clandestinely. In short, the committee has been under considerable pressure to come up with regulations which would allow some of the work to be resumed, and which would at least remove the uncertainty. As one committee member said, if the committee failed to draft regulations at its December meeting, "the dam would be likely to break".

Thus, early in July, a subcommittee under the chairmanship of David S. Hogness, of Stanford University, drafted a set of regulations for consideration by the full committee at a meeting held in Woods Hole later that month. The Hogness version offered some stringent controls which, in most respects, conformed to the Asilomar guidelines, but which were weakened in a few key places at the Woods Hole meeting. It was those changes which have caused the committee most of its problems, and which led to the meeting in La Jolla.

The Woods Hole draft was to have been the committee's final report, but when it was circulated around the scientific community, it encountered so much criticism—including adverse reaction from two committee members, Roy Curtiss III of the University of Alabama, and Stanley Falkow of the University of Washington (who was not present at the Woods Hole meeting)—that it was never published in final form. Instead, the committee chairman, DeWitt Stetten, Deputy NIH Director for Science, appointed another subcommittee under the chairmanship of Elizabeth Kutter of Evergreen State College, Washington, to draft an alternative set of regulations for the committee to consider. The Kutter draft, which was completed in November, was in some respects more restrictive than either the Hogness or Woods Hole versions.

Among the criticisms levelled at the Woods Hole draft was a statement signed by some 50 participants at a conference held in August at Cold Spring Harbor, who complained that it "appears to lower substantially the safety standards set and accepted by the scientific community as represented by the meeting at Asilomar". And another particularly influential critic of the draft was Paul Berg, who wrote in a letter to Stetten that the restrictions proposed for some types of experiments are "marginal and inadequate considering the risks the document itself concedes"; he suggested that in one particular case, the regulations "are very likely to draw the charge of self-serving tokenism". Some critics took the opposite view, however, suggesting that the Woods Hole regulations were so strict as to constitute unwarranted infringement on academic freedom.

Thus the committee met here in a situation which had all the ingredients of a possible show-down, but it nevertheless conducted its business with little animosity. The entire committee turned up (15 members including the chairman), together with six special consultants who included Berg and Maxine Singer, a virologist from the NIH who was an organiser of the Asilomar conference.

On the first day of the meeting, the committee approved, by a series of close votes, restrictions on some types of experiment which differed little from the Woods Hole draft, and which would inevitably have drawn considerable criticism for being too lax. But on the second day, it went back over some of those recommendations and, by equally close votes, tightened the regulations considerably, so that in some instances they are even more stringent than the Asilomar guidelines.

It is difficult to know exactly what made a few committee members change their votes, but one member indicated privately that after considering the matter carefully, he decided that in cases where there is disagreement, the committee should, as a matter of policy, adopt the more stringent option. It is always possible to lower the restrictions at a later date, he suggested, if evidence becomes available to suggest that the risks have been overstated. Another consideration was the practical one that if the committee adopted regulations which meet with criticisms for being too lax, then it was entirely possible that the matter could be taken out of the hands of scientists, and the technique regulated by legislation—a possibility which clearly bothers many people because it smacks of political infringement on academic freedom.

The committee's move to adopt strict regulations was made easier by an important development. A week or two before the meeting, Roy Curtiss, whose laboratory has been working full-time since the Asilomar conference on the problem of constructing a genetically crippled strain of the *E. coli* K12 bacterium, finally produced a strain which seems to meet all the requirements. He has tested it in rats, and found that it does not survive in the intestine, and after some more tests are completed, he believes that it will fit the bill. Similarly, at least two strains of bacteriophage lambda have passed

Figuring the risk

THE hazards associated with cloning recombinant DNA molecules can only be speculated about, since there is no experimental evidence to prove or deny that they exist. Thus the NIH advisory committee was attempting to write regulations based largely on guesswork about the likelihood that, for example, a bacterium or virus bearing genes transplanted from another organism could pose a danger to animals or plants — a situation, as DeWitt Stetten, the committee chairman points out, "in which Lloyd's of London refuses to write insurance".

The committee therefore unanimously approved a resolution urging the NIH to sponsor a potentially hazardous experiment, in maximum physical containment facilities, to gain some data on the likely extent of the risks. The experiment, which was suggested by Sydney Brenner of the MRC Laboratory for Molecular Laboratory, Cambridge, would involve splicing genes from a polyoma virus (a mouse tumour virus) into a bacterial plasmid, cloning the recombinant molecule in *E. coli*, and infecting young mice with the modified bacterium.

The idea would be to monitor the mouse's serum to determine whether antibodies to polyoma virus are formed. If they are, it would indicate that the transplanted genes are working, and are being transmitted into the animals' blood stream, in which case there is a real danger that many of the postulated risks exist. If no serum antibodies are formed, it would indicate that at least in this case, the genes are not being expressed in the animal's system, which would suggest that some of postulated hazards are less plausible. There is a danger, however, that such a negative result could be over-interpreted as suggesting that recombinant experiments are inherently safe.

If the experiment is carried out, it would be performed in maximum containment facilities at the NIH, which easily meet the P4 requirements, and it would be conducted by an eminent virologist.

preliminary tests which indicate that they are crippled sufficiently to provide an extremely high biological safety barrier. Again, they need more rigorous testing before such expectations can be confirmed. The committee was thus able to write regulations specifying the use of crippled micro-organisms for many types of experiment, with a high expectation that the strains would soon be available.

This, in short, is what the committee finally decided. It has defined a series of conventional, physical safety levels, designated as P1 to P4, ranging from simply the use of standard microbiological techniques (P1), to specially designed facilities with negative air pressures, isolated by airlocks, with showers at each exit, and so on (P4). It has also defined three biological safety barriers, designated EK1, EK2 and EK3 for experiments in which genes are inserted into *E. coli* K12 for cloning.

At the risk of slight distortion by over-simplification, the three levels are defined as follows

- EK1 This is the lowest level of biological containment. It requires use of standard *E. coli* K12, which, according to several tests, is naturally a feeble strain. The genes to be cloned would first be spliced on to either a bacterial plasmid (a ring of DNA which replicates inside the bacterium independent of the bacterium's own DNA) or on to the DNA of bacteriophage lambda which would then infect the bacterium.
- EK2 This level of containment involves the use of crippled strains of *E. coli* or bacteriophage lambda for which laboratory tests have indicated that fewer than 1 in 10^8 cloned pieces of DNA would survive outside the laboratory.
- EK3. This level is essentially the same as EK2, except that it has been rigorously tested by field trials in man, primates or plants.

At present, only the EK1 level is available, but if the crippled *E coli* produced by Curtiss, or the crippled bacteriophage lambdas (the most promising of which has been produced by Fred Blattner of the University of Wisconsin) meet the requirements, they would constitute an EK2 system. The committee itself will examine the test data and certify whether any of those strains do, indeed, meet the requirements for EK2. The tests needed for EK3 have not yet been undertaken, and they could take several months to complete.

The committee's chief task was to assign various levels of containment to different types of experiments. First, however, it specified some experiments which are considered potentially too risky to undertake at all. The committee recommended, for example, that DNA from highly pathogenic organisms (class 3, 4 and 5 agents according to the classification adopted by the Center for Disease Control, CDC) should not be cloned, that genes which cause the production of highly toxic compounds (such as diphtheria toxin) should not be spliced on to other pieces of DNA, and so forth. There was little disagreement over the need to outlaw such experiments.

The sharpest areas of disagreement involved the assignment of so-called shotgun experiments to various safety levels. Shotgun experiments involve chopping up an organism's whole genome (or at least a substantial part of it) and splicing the fragments into plasmids or into the DNA of a bacteriophage for insertion into *E. coli.* Such experiments are considered to be hazardous since they involve cloning unknown fragments of DNA, and those involving DNA from primates or man are considered the riskiest of all since the cloned DNA may contain genes from cancer viruses. The committee's final recommendations are contained in the accompanying tabulation; they are mostly more stringent than the Woods Hole draft, and they are all at least as strict as the Asilomar guidelines.

At least one novel concept emerged from the discussions on shotgun experiments. It was agreed that since embryonic cells are less likely to have been infected by viruses, DNA taken from embryonic tissue is likely to be safer to use in a shotgun experiment than DNA from adult tissue. Thus, such experiments were assigned a lower category. Moreover, it was also agreed, after considerable discussion, that if the DNA to be cloned has been purified so that at least 99% of it is a known, non-harmful gene, the cloning can be performed at a lower physical containment level.

Another area which caused considerable discussion was the containment levels necessary for a class of experiments involving the splicing of DNA from various organisms on to the DNA of animal viruses, and infecting cell cultures with the resulting hybrid virus. In other words, the virus is used as a vehicle for cloning the transplanted genes. Two different viruses, a monkey

THE committee defined four levels of physical containment, designated, in order of increasing severity P1 to P4, and three levels of biological containment, EK1 to EK3 (see text) and assigned experiments to them on the basis of potential risk. The following is a tabulation of the committee's proposals showing containment levels for various sources of DNA. It is an unofficial version which should be checked against the committee's final report when it is published.

a. Shotgun experiments using *E. coli* as the host	
Non-embryonic primate tissue	P3+EK3 or P4+EK2
Embryonic primate tissue	P3+EK2
Other mammals	P3+EK2
Birds	P3+EK2
Cold-blooded vertebrates, non-embryonic	P2+EK2
embryonic	P2+EK1
Invertebrates and lower plants	P2+EK1
Higher plants	P2+EK2
Higher plants which produce toxins	P3+EK2
Prokaryotes which exchange genes with *E. coli*	
Class 1 pathogens (for example, enterobacteria)	P1+EK1
Class 2 pathogens (for example, *S. typhi*)	P2+EK2
Higher risk pathogens	Banned
Prokaryotes which do not exchange genes with *E coli*	
Non-pathogens	P2+EK1
Low risk pathogens	P3+EK2
Moderate pathogens	P3+EK3 or P4+EK2
Higher pathogens	Banned
In all above cases, if the DNA is at least 99% pure before cloning, the physical containment level can be reduced one step.	
b. Cloning virus genes in *E. coli*	
Animal viruses	P3+EK3 or P4+EK2
Plant viruses	P3+EK1 or P2+EK2
c. Eukaryotic plasmid or organelle DNA cloned in *E. coli* if DNA is 99% pure, and consists of harmless genes	
Primate tissue	P3+EK1 or P2+EK2
Others	P2+EK1
If DNA is impure, then shotgun conditions apply	
d. Animal virus vectors	
Defective polyoma+DNA from class 1 virus (CDC classification) or non-pathogen	P3
Defective polyoma+class 2 virus	P4
if virus DNA known to be non-harmful	P3
Defective SV40+class 1 virus or non-pathogen	P4
Defective SV40+non-pathogenic DNA from either prokaryotes or eukaryotes	P3
Defective SV40 or polyoma lacking late genes+prokaryotic or eukaryotic DNA provided no virus particles are present	P3

virus called SV40 and a mouse virus called polyoma virus, are being used in such experiments

The committee eventually assigned all such experiments to very high physical containment facilities, according to a set of guidelines drawn up by a group of animal virologists, including Berg and Singer, who were present at the committee meeting as consultants or observers. Berg apologised for bringing in such complicated proposals at a late stage in the proceedings. Another committee member reflected that such last-minute proposals indicate the speed with which the field is moving, and that he would like to keep it moving, a statement which Berg labelled "self-serving".

After the meeting was over, Berg said that he was happy with the final draft, and indicated that it had tightened the restrictions in areas which bothered him in the Woods Hole recommendations. He said that he believes the final recommendations "have incorporated the spirit of Asilomar".

Although the committee has demonstrably accepted strict regulations, it is nevertheless open to the charge that its deliberations have had no input from the general public. The committee consists entirely of scientists, some of whom are using, or planning to use, the cloning technique, a fact which has already raised suggestions of conflict of interest. But the stringency of the regulations is such that those criticisms can easily be blunted.

The committee's report should be available in a few weeks. The recommendations will go to the Director of the NIH, who will probably accept them, and they will eventually be used by the NIH in awarding grants for such research. ☐

Concorde Mark II?

WITH Concorde due to commence passenger services on January 21, and a decision awaited from the US Government clearing it for the Atlantic run, the prospect of a "second-generation" supersonic airliner was aired last week by the Vice-chairman of Rolls-Royce (1971), Mr Kenneth Wilkinson, when he delivered the annual Wright Memorial Lecture to the Royal Aeronautical Society in London.

Concorde, he said, was expected to demonstrate the value of supersonic air transport for a sufficiently important segment of the market to justify a second-generation version for service in 10 to 15 years' time. The indications were that such an aircraft could be produced profitably with "acceptable noise characteristics and economic operation at less than first-class fares".

Letter from Egypt

from Salah Galal, Cairo

ENERGY needs for Egypt's projected population of 220 million in the year 2100 would demand the construction of two Aswan High Dams a year; water requirements demand the equivalent of two extra Nile Rivers, making desalination essential. Only nuclear energy offers the means to fulfil these demands. The agreement signed with Washington during the recent visit of President Sadat to the United States involves two light water power reactors, using boiling water if Westinghouse is brought in or pressurised water if General Electric is.

The International Atomic Energy Agency (IAEA) and the General Egyptian Electricity Corporation (GEEC) estimate that Egypt will be using nuclear energy to cover 50% of its energy needs for electricity and desalination by the year 2000 and will need 20,000 tons of uranium concentrate for the reactors—the equivalent of 400 million tons of oil.

Cumulative uranium requirements for power and desalination programmes were found to be in the range of 19,000–25,000 tons in case of a heavy water reactor (HWR) strategy, and 30,000–40,000 tons for a light water reactor (LWR) strategy. In the year 2000 alone, the demands are expected to be 2,000–2,500 tons and 3,200–4,000 tons, respectively.

These needs make intensive uranium exploration, coupled with a suitable reactor policy, essential to the development of a local nuclear fuel cycle technology. These matters are now under study, but the first atomic power plant (EFAPP), together with the two American reactors which will be constructed at Sidi Kreir near Alexandria and should be operating in 1982, will be of the LWR type. No final decision on subsequent nuclear power stations has been taken.

Estimates of the water demands for agricultural, municipal and other purposes suggest a deficit of 2×10^9–3×10^9 m^3 by 1980, increasing to 17×10^9–18×10^9 m^3 by 2000. Nuclear desalting would make up part of the shortfall—about 4×10^9–5×10^9 m^3—in the year 2000. The power requirements will be around 9,000 MWe This means a nuclear programme 50% greater than that projected by the GEEC for nuclear power is needed.

- Ahmed Sultan, Egypt's Minister of Electricity visited Paris recently to discuss the purchase of two more nuclear reactors, which will be in addition to the two American reactors. During last week's state visit to Egypt of the French President, Valéry Giscard d'Estaing, the two countries signed an agreement providing for the construction of a 600 MW nuclear power plant in the Nile delta area. The plant is due to go into operation in 1984.
- The Animal Bioclimatology Unit of the Egyptian Atomic Energy Establishment has decided to take part in agriculture and livestock projects which might help remedy deficiencies in the country's food production It is believed that radioisotopes can be used to improve productivity in hot desert areas: Professor Taymour Kamal, who heads the unit, has used radioisotopes and tracer techniques to develop new methods for predicting heat tolerance in farm animals. Young animals selected for hot areas would be expected to have high productive potential after growing in the Sahara's hot climate. Kamal has criticised conventional heat tolerance indices as unrelated to the productive performance animals, and says his tracer methods measure directly the animal's loss in body fat and lean mass.

One of his methods is based on a 3-minute count of radioactive ^{40}K occurring naturally in the animal's body before and after exposing it for 3 days to a temperature of 32 °C. The loss of lean tissue can then be calculated since the radioactive potassium exists in a definite and constant ratio to all natural potassium, which is found in fairly constant concentration in lean tissue Young animals which show little or no tissue destruction during the heat exposure are considered heat tolerant and suitable for raising in hot areas.

A second tracer method measures both the lean tissue loss and the fat loss. This is based on a determination of the total body water of the animal before and during the 3 days of heat exposure. These figures are subtracted from the corresponding live body weights to obtain the total body solids, mostly fat and protein, before and during heat stress. The animals screened have shown great variation in the percentage loss of body solids ranging from 0.2% to 24.5%. Those animals which show a small loss are considered heat tolerant. Other tracer methods for heat tolerance are still under investigation in the unit.

SCIENCE COLLEGE
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CALCUTTA

Mr Wilkinson gave a figure of £5,500 millions for development costs. The aircraft would sell for about £65 millions, and use a turbofan derivative of the Rolls-Royce Olympus 593 engine presently powering Concorde. But, he added, there should be only one design This would mean another joint international venture, which might include the Soviet Union as well as the USA.

Rolls-Royce meanwhile sucessfully completed a £100 millions deal at the weekend with the Chinese National Technical Import Corporation under which China will, over the next two years, import a limited number of Spey turbofan engines manufactured in Britain. Rolls Royce will supply the Chinese with the expertise they need to manufacture the engines under licence. The licence itself covers both military and civil versions of the turbofan, and Rolls-Royce are understood to be supplying afterburners along with the complete engines. With China already heavily committed to the Hawker Siddeley Trident, which is fitted with Spey engines, the reinforcement of the link with British aerospace technology encouraged speculation about the status of options China has on three Concordes.

● European cooperation on another project, the Multi-Role Combat Aircraft (MRCA), received a bit of a boost with the signing late last week of a contract for the initial investment required to start production. The contract, worth several million pounds, was signed by the NATO Military Management Agency (NAMMA), which runs the programme for the three governments involved, West Germany, Italy and the UK. It authorises a three-nation airframe consortium to start work. The production go-ahead itself, for more than 800 aircraft, is expected next year. ☐

Pollution proposals

by Allan Piper

A PROPOSAL for blanket legislation to standardise pollution levels within the European community was modified last week when EEC environment ministers meeting in Brussels reached a compromise which will allow member states some flexibility in the administration of pollution controls. Instead of observing pollution limits based on measurements of waste levels at the source of emission, members will be able to opt for a scheme whereby pollutant concentrations are monitored after dispersion into the environment. In considering each case on its individual merits the scheme will allow some countries to dump more toxic waste than others.

The agreement represents something of an achievement for the UK Government which, with the backing of its Royal Commission on Environmental Pollution, had opposed the Commission's proposals for uniform standards. With an alternative available, the UK can continue its present policy of using "quality controls" to restrain the dumping of toxic wastes like cadmium and mercury.

The wrangle between the UK and its EEC partners began when the environment ministers last met in Luxembourg in October. The UK then rejected the Commission's call for the introduction of so-called 'emission norms'—uniform standards based on the levels of toxic waste actually produced from any source. The UK argument in favour of quality controls was based on the view that EEC member states had diverse environments which could accommodate widely differing concentrations of pollutants. The northern Atlantic off the Scottish coast, the argument ran, was more easily able to disperse a given level of toxic substances than the coastal waters of the Adriatic.

The latest agreement, which came as a surprise, means that while the Commission will stand by its original proposal for emission norms, individual members can opt for quality control. But there is a caveat. They must satisfy the Commission that their controls are equally effective in maintaining levels below the prescribed limits. The UK Department of the Environment is confident that Britain will have no difficulty in meeting this condition.

The quality controls will be re-examined by the Council every five years. Meanwhile the finer details of the two systems will be drafted. The Commission has two years to complete its proposals which will then go before the Council for approval. Once approved, the member states will begin arrangements to implement the necessary legislation. Since this will probably take about five years altogether, the UK's present pollution policy is unlikely to come under further EEC scrutiny for about a decade.

The main opposition to the idea of control by quality norms came from West Germany and Holland, both of which have to contend with the peculiarly acute pollution problems posed by the Rhine. Their antagonism apparently stemmed from their concern that favourably located countries like Britain should not gain unfair commercial advantages by not being required to bear the individual cost of complying with uniform legislation. UK industry could have been faced with a bill estimated at £750 millions had the original community proposal remained unmodified. ☐

Kovalev sentenced

from Vera Rich, London

ON the very day that he was due to receive his Nobel Peace Prize, Dr Andrei Sakharov was in Vil'nyus unsuccessfully seeking admission to the trial of Dr Sergei Kovalev, a biologist and member of the banned Moscow group of Amnesty International.

Dr Kovalev was arrested a year ago on various charges of "anti-Soviet agitation and propaganda" and "slandering the Soviet Union". These included the production and distribution of *samizdat* journals and of Solzhenitsyn's *Gulag Archipelago*, and also the circulation of the illegal *Chronicle of the Lithuanian Catholic Church*, which provided the reason for holding the trial in Vil'nyus rather than Moscow. Dr Kovalev is not a Lithuanian, nor, so far as is known, has he any connection with the Catholic Church. His only connection with the *Chronicle* seems to have been that when his flat was searched, a few pages from a Russian-language edition of it were found among his collection of *samizdat* materials.

If it was hoped that by changing the venue the trial might attract less notice, this was largely vitiated by Dr Sakharov's presence, which helped to ensure that world attention was focused on the Vil'nyus courtroom. Also present in Vil'nyus, and unable to gain admission to the courtroom, was physicist Yurii Orlov, a friend of both Kovalev and Sakharov. Kovalev was sentenced to seven years' jail with hard labour and three years' exile.

During the Vil'nyus hearing the case was recalled of another dissident, the Ukrainian mathematician Leonid Plyushch, held in a penal psychiatric institution on account of his dissident and "separatist" views. Plyushch has been a cause celebre for all those who have campaigned against alleged misuses of psychiatry for political ends. Recently, after a massive rally and campaign in France, in which left-wing and communist organisations played a considerable part, the Soviet authorities promised Mrs Plyushch that her husband would shortly be set at liberty. No definite information as to a release date has been given, but according to dissident sources, one of the psychiatrists who had been treating Plyusch was called to give evidence against Dr Kovalev and stated that he had lied when he wrote that dissidents were being confined in mental hospitals as a means of political repression.

Meanwhile Mrs Sakharov has reportedly criticised the western press for emphasising the difficulties of Soviet intellectuals instead of the idea that "an innocent man ought to have nothing to fear in our country". ☐

correspondence

New president of Soviet Academy

Sir,—In your recent article "Appointment to Soviet Academy threatens autonomy" (December 4, page 377), your correspondent's interpretation of the changes in the Academy leadership was superficial and prejudiced. The conclusion that the replacement of Academician V. A. Kotel'nikov by Academician A. P. Alexandrov indicates "a greater degree of Government supervision of the Academy's work in future" was made, first, because Alexandrov was introduced at the Academy meeting by Politburo member M. A. Suslov and not by somebody from the Academy's own ranks, and, second, because Alexandrov himself is a member of the Central Party Committee Plenum.

Both aspects are irrelevant in any assessment of the Academy's future. The replacement had to be made by a high Party or Government official because the Academy is not and never has been independent; it is a State scientific establishment.

With the position of the chairman of the "Ideological Commission"—which includes the Party's department on science and education—vacant more than a year already, M A. Suslov (as top Party ideologist) is now the highest official responsible for science and education There is also nothing sinister in the fact that Alexandrov is a member of the Central Party Committee. The most liberal Russian poet, writer and editor Alexander Tvardovsky was also a member of the Central Party Committee

I consider that the election of Alexandrov now is a concession from the Government, a move to meet the demand of the Academy for a more popular and prominent figure. If this is so, the new election is a liberal decision, and does not reflect an increase of political pressure Moreover, regarding Alexandrov himself, it should be noted:

- When, during Khrushchev's time, classical genetics was still forbidden and Lysenko reigned supreme, the only place where large-scale, serious genetic research was possible was the big biological division of the Kurchatov Institute of Atomic Energy headed by Alexandrov—a division created specifically for confrontation with Lysenko pseudogenetics.
- When in 1966 Alexander Solzhenitsyn decided to give public readings from his banned novels, the only place where he was able to do this was the Kurchatov Institute. The hundreds of scientists there warmly welcomed the writer, and Alexandrov was reprimanded for taking this liberty.
- When in 1970 I was put by force into a mental hospital for political reasons, academicians who acted on my behalf, strongly and voluntarily, included Alexandrov Indeed, he was the most influential among them, and the only one who did not know me personally, yet he spoke strongly in support of my immediate release
- Alexandrov's predecessors, Kotel'nikov and M. V. Keldysh, signed a letter to *Pravda* on August 29, 1973, against A Sakharov. Alexandrov did not.

The Soviet Academy, being a state body, has never really been autonomous But it has moral authority and political influence which derives mostly from the personal prestige of academicians and their scientific stature It also stems from the courage of some of the Academy's best people in their struggle for scientific integrity and scientific and human rights This struggle has not always been successful, but there have been several cases in recent years when the majority of the Academy has strongly opposed Government pressure on certain issues.

Your correspondent was also wrong to predict a short term for Alexandrov merely because of his age. The position of president is not tenured for life; it is subject to re-election every five years. The only president who was not able to complete this minimum term was the young and bright Sergei Vavilov, who died from a heart attack in 1951.

Yours faithfully,
Zhores A. Medvedev

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for Medical Research,
London, UK*

Multiplying ventures

Sir,—During the past fortnight I have received three communications on the formation of new groups The first of these is from the Society of Experimental Biology, it refers to the formation of a neurobiology group. The second one comes from the secretariat of a newly formed European Neuroscience Association. The third one is a communication concerning the first meeting of a newly formed European Society of Neurochemistry.

I am already a member of the International Brain Research Organisation (IBRO) and I believe I am also a member of the International Society for Neurochemistry. I also frequently attend meetings of the Neurochemistry Group which meets under the auspices of the Biochemical Society.

Faced with this plethora of new ventures one is tempted to ask the organisers: "Is your venture really necessary?" It seems to me as if in this field there is a development of much overlap Is there no roof organisation that could be consulted when such new societies are formed? The European Neuroscientists' missive contains the threat that "a fee structure should be set up by the first Council for individual membership". The European Neurochemists require a registration fee of not more than £15 for participant members of their first meeting.

For one whose interests happen to bridge the areas of several or all of these bodies considerable monetary expenditure seems to be involved. Surely this multiplication of organisational effort is not in the spirit of the times

Yours faithfully,
H. Blaschko

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Journal guidelines

Sir,—Why does Professor Ziman think it a populist folly to disclose the names of referees to authors? In the *Journal of Aerosol Science*, **1** (1), 1970, I wrote "A feature of the new journal is that no cloak of mystery will shroud those who accept, reject or accept subject to modification the papers which are submitted to it. There are three reasons for this unorthodox exposure of the referee. First, the authoritarian age is over, second, the standard of critique will be raised and lastly, it is hoped, encouragement and benefit will result to authors"

Six volumes of editorial experience confirm this statement. What has Professor Ziman to offer in support of his assertion?

Yours faithfully,
C N Davies

*Department of Chemistry,
University of Essex, UK*

news and views

Chemical identification of a natural opiate receptor agonist in brain

from Leslie Iversen

MORPHINE and related opiates interact with highly specific receptor sites which are present in greatest abundance in certain circumscribed areas of brain, particularly in association with neural pathways involved in the perception of pain (see Snyder, *Nature*, **275**, 185; 1975). None of the established chemical transmitter substances in brain seems to interact strongly with the opiate receptors, suggesting that the mode of action of these drugs does not involve any of these known mechanisms. This has led to renewed interest in the hypothesis that the brain may contain a hitherto unidentified substance which normally acts as the endogenous activator of the opiate receptors, a possibility which now appears to have been substantiated.

Hughes (*Brain Res.*, **88**, 295; 1975) and Hughes *et al.*, (*Life Sci.*, **16**, 1753; 1975) obtained evidence for the existence of an endogenous factor with the properties of morphine in brain by testing brain extracts on two biological preparations that were known from previous studies to respond specifically to opiate drugs. These tests are based on the ability of morphine and related drugs to block electrically evoked contractions of mouse vas deferens or guinea pig intestine. In each case a crucial item of evidence was that the effects of morphine and of the morphine-like compound in brain extracts could be blocked by low concentrations of specific morphine antagonists such as naloxone. Using these test systems as bioassays, it was possible to develop an extraction and purification scheme for the isolation of pure material with the properties of morphine from pig brain. This substance was called "enkephalin" and had a molecular weight of approximately 1,000; it rapidly lost biological activity if exposed to proteolytic enzymes, suggesting that it might be a peptide. A similar substance could be shown to be present in extracts of cow, guinea pig, rabbit, rat and mouse brains. In the pig brain, enkephalin was unevenly distributed, being most concentrated in regions known from previous work on other species to contain the highest density of opiate receptor sites.

Another method for detecting the presence in brain extracts of a substance acting like morphine is to measure its ability to displace the specific receptor binding of radioactively-labelled opiate drugs such as etorphine or naloxone to brain membrane preparations *in vitro*. This approach has been used successfully by Terenius and Wahlstrom (*Acta physiol. scand.*, **94**, 74; 1975) and by Pasternak, *et al* (*Life Sci*, **16**, 1765, 1975), who have also described the existence of a morphine-like substance in extracts of calf, rabbit and rat brain. The compound has a molecular weight of approximately 1,000 and is susceptible to degradation by proteolytic enzymes, suggesting that it may be a small peptide. It has a regional distribution in brain similar to that previously observed for opiate receptor sites. Terenius and Wahlstrom (*Life Sci.*, **16**, 1759; 1975) also obtained evidence for the existence of a similar substance in samples of human cerebrospinal fluid. The potencies of morphine agonists (mimetic drugs) in the radioactive ligand binding assays are decreased by the presence of low concentrations of sodium ions in the incubation medium, whereas the potencies of antagonists are increased by sodium ions (Pert and Snyder, *Molec. Pharmac.*, **10**, 868; 1974). The ability of the morphine-like factor to displace radioactive ligands was greatly decreased by adding sodium chloride suggesting that the substance is a relatively pure agonist at opiate receptors.

A very important advance in this field is now described by Hughes *et al.* (page 577 of this issue). They have been able to complete the chemical identification of enkephalin previously purified from pig brain. Using conventional methods of amino acid sequence analysis and independent mass spectrometric analysis they report that porcine enkephalin consists of a mixture of two pentapeptides: H-Tyr-Gly-Gly-Phe-Met-OH (Met-Enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-Enkephalin), in a ratio of approximately 3 : 1. Both peptides have now been synthesised, and the synthetic materials shown to mimic the actions of naturally occurring enkephalin on the guinea pig ileum and mouse vas deferens preparations. Met-Enkephalin is effective at very low concentrations on the mouse vas deferens, in which it is about twenty times more potent than the powerful opiate agonist normorphine. It is also some three times more potent than morphine in a ligand binding assay. Leu-Enkephalin is somewhat less potent than the methionine analogue.

It remains to be seen whether enkephalin as described by Hughes *et al.* corresponds to the endogenous morphine-like factors of Terenius and Wahlstrom and Pasternak *et al.* although this seems very likely. The relationship, if any, of these small peptides to the somewhat larger peptide described by Teschemacher *et al.* (*Life Sci.*, **16**, 1771; 1975) and by Cox *et al.* (*Life Sci.*, **16**, 1777; 1975) in extracts of bovine pituitary glands is also obscure. These authors reported that impurities in commercial preparations of the pituitary peptide ACTH and a substance present in extracts of pituitary had acted like morphine in the guinea pig ileum, mouse vas deferens and ligand binding assays. These findings may perhaps relate to the observation of Terenius *et al.* (*Eur. J. Pharmac*, **33**, 395; 1975) that various synthetic ACTH-like peptides have properties similar to morphine. It is possibly significant that the amino acid sequence of Met-Enkephalin is also found in residues 61-65 of the pituitary peptide β-lipotropin, which is related to ACTH.

The identification of enkephalin and its availability in synthetic form will allow rapid progress to be made in answering the many questions that remain. Does enkephalin have analgesic properties? (As a corollary, is enkephalin even capable of penetrating into the brain from the bloodstream after systemic administration?) Why are there apparently two peptides with similar biological properties? Are they always found together in different brain regions? Perhaps the most important practical question is whether enkephalin offers the long sought approach to the development of a non-addictive analgesic. Kosterlitz and Hughes (*Life Sci.*, **17**, 91; 1975) have described some preliminary evidence in favour of this view. They point out that enkephalin has some unusual pharmacological properties resembling those seen in certain recently developed synthetic drugs of the benzomorphan class, which seem to be free of addictive properties in monkey tests. These properties are, first, a relatively rapid rate of onset and short duration for the effects on opiate receptors in isolated test organs and second that the actions are less readily blocked by standard antagonist drugs such as naloxone. To antagonise a response of given magnitude requires about a four times higher concentration of naloxone when enkephalin is the agonist than when conventional agonists such as normorphine are used.

There will also undoubtedly be rapid progress in establishing radioimmunoassay methods to measure enkephalin in brain, and possibly immunohistochemical techniques to elucidate its precise cellular localisation in different brain regions. This information should help to clarify the possible physiological role of enkephalin as a neurotransmitter or neuromodulator substance associated with pain pathways in brain. □

A photosynthetic structure

from A. G. Lee

READING the literature on photosynthesis is almost certainly bad for the health. To be told that in the green photosynthetic bacteria, exposure to light causes a flow of electrons from Chl-660 to Chl-770 then to P840 and finally to C551, C553 and C555 does little to reassure one, especially when informed that Chl-770 is also called B810, that P840 could be an aggregated form of Chl-770 and that C551 and C555 also masquerade under the names C419 and C422 respectively. These names of course reflect the fact that the physical and chemical identity of the species are generally unknown, and that they have been recognised only from changes in absorption spectra. For any satisfactory model of photosynthesis, it will be necessary to translate these labels into actual chemical species present within the photosynthetic system. In a paper by Fenna and Matthews in this issue of *Nature*, (page 573), a start is made towards this goal.

In the green bacterium *Chlorobium limicola* (once called *Chloropseudomonas ethylica*) the photosynthetic apparatus is contained within egg-shaped vesicles, about 50 nm wide and 150 nm long, arranged as a layer along the inside face of the cytoplasmic membrane. In this it differs from the purple photosynthetic bacteria, where the photosynthetic membrane is continuous with the cytoplasmic membrane. The photosynthetic apparatus in the green bacteria also differs in that they contain an extrinsic chlorophyll-containing protein which is water soluble and can be removed by changes in buffer strength and *p*H: the chlorophyll-containing proteins in purple bacteria and plants are firmly attached to membranes and can only be removed by detergent treatment. Because it is water soluble, it has been possible to purify and crystallise this chlorophyll–protein from *C. limicola*, and it is the structure of this protein that is reported by Fenna and Matthews.

Although the bulk of the chlorophyll in *C. limicola* is chlorobium chlorophyll (also called bacterioviridin or Chl-660), there is present as a minor component bacteriochlorophyll (Chl-770, also called, you will remember, B810), and it is bacteriochlorophyll that is contained in the water-soluble protein. Since the amino-acid sequence of the protein is unknown, the X-ray crystallographic studies only give information about the α-carbon backbone, but the structure is sufficiently defined to show the basic protein conformation.

The protein consists of three identical subunits, each containing in its centre seven bacteriochlorophyll molecules arranged in an apparently irregular fashion. The structure of each subunit is described as a distorted hollow cylinder, with one end of the cylinder possibly being open. The interesting and unique aspect of the structure is the hollow cavity within each cylindrical subunit, with the bacteriochlorophyll molecules being bound to the 'walls' of this cavity. The binding of the bacteriochlorophyll molecules to the protein is partly due to interactions between the phytyl chains and what are probably hydrophobic residues facing towards the centre of the subunit. There are also, however, extensive interactions involving the Mg ions of the porphine rings, either through hydrogen bonding between the protein and a water molecule coordinated to the Mg, or through a direct interaction between the Mg and a suitable coordinating group of the peptide. Hydrogen bonding is also possible between some of the substituents of the bacteriochlorophyll and the protein. Although there seems to be no direct contact between the porphine rings, the average centre-to-centre nearest neighbour distance is only 12 Å, so that efficient energy transfer is possible within each subunit.

If the structure of this particular chlorophyll-containing protein is at all typical, then it makes the derivation of molecular models from absorption spectra seem an almost impossible task, since although the planes of the seven porphine rings in each subunit are close to being parallel, there is no obvious pattern to their relative orientation. The question as to how important are the chlorophyll–proteins generally in the photosynthetic process still remains open however. The actual role of the bacteriochlorophyll–protein for example is still uncertain: although it has been suggested that it acts to transfer excitation from the antenna chlorobium Chl-660 to the reaction centre P840, it has not yet been demonstrated that photosynthesis fails to occur in its absence. The fact that it is water soluble also makes it highly atypical. The isolation of chlorophyll-containing proteins from other photosynthetic systems requires the use of detergents, and it is not then at all clear whether the isolated complexes correspond to species actually present in the membrane. Thus although membrane-bound proteins are generally isolated in detergent solutions as lipid–protein complexes, this does not mean that they were present in that form in the membrane: the presence of an organised lipid bilayer in the membrane means that hydrophobic surfaces of the membrane proteins are isolated from the aqueous phase, whereas in the detergent solution, a specific lipid–protein interaction is necessary to provide the same protection. The observation that the majority of the chlorophyll in a photosynthetic membrane is found associated with protein after detergent treatment similarly does not mean that it was the same in the membrane. In fact, it is known that chlorophyll incorporated into a lipid bilayer in the absence of any protein will adopt an aggregated structure analogous to that proposed for antenna chlorophyll (Strouse, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 325–328; 1971). Nevertheless, it is obvious that chlorophyll in the reaction centres must be closely associated with protein, and the structure reported by Fenna and Matthews shows something of the complexity that might be expected. □

Site-directed tumour chemotherapy

from a Correspondent

THE interesting idea of site-directed killing of tumours by cytotoxic agents coupled to molecules with affinity for the target tissue was first suggested seventy years ago by Paul Ehrlich. Apart from the obvious and probably surmountable difficulty of obtaining suitable tissue-, or better tumour-specific agents (antibodies for example) for use in treatments of this kind, there is the problem of getting to the cancerous site sufficient of the cytotoxic substance to cause an effect. Exceptionally potent toxins are required therefore that can kill cells when present in very low concentration. Diphtheria toxin is one such agent The toxin is a protein of molecular weight 62,000 which is synthesised in high yield by certain lysogenic strains of *Corynebacterium diphtheriae* after infection with bacteriophage. This killing effect is mediated by the strong inhibition of protein synthesis, probably by the catalytic addition to aminoacyl transferase II of ADP-ribose from NAD. Since protein synthesis in a fairly wide range of cells is inhibited by the toxin *in vitro*, the fact that for example the growth of mouse L cells is not prevented, means that the cytotoxic action requires binding of the toxin to surface receptors as an obligatory first step. The toxin may be cleaved by some proteases into two polypeptide fragments A and B. The B fragment binds to cell surface receptors and fragment A inhibits polypeptide elongation. Receptors are present on human cells but not mouse cells and although these are very few (25–50 per cell) this is sufficient to make the cells sensitive. Perhaps as few as 1 or 2 toxin molecules delivered to the cell surface are cytocidal.

Earlier experiments (Moolton *et al.*, *J. natn. Cancer Inst.*, **49**, 1057–1062; 1972, Philpott *et al.*, *Surgery*, **73**, 928–935; 1973) showed that diphtheria toxin coupled to antibodies directed against dinitrophenyl or mumps virus selectively killed cells bearing these antigens when the appropriate conjugate was added to the culture medium. *In vivo* the toxin–anti-dinitrophenyl antibody conjugate protected to some extent hamsters concurrently challenged with viable DNP-coated sarcoma cells. In their latest paper Moolten *et al.* (*J. natn Cancer Inst.*, **55**, 473–477; 1975) have applied sublethal doses of diphtheria toxin conjugated to antibodies directed against SV40-associated cell surface antigens, to hamsters bearing SV40-induced tumours with some encouraging results Treatment of hamsters with a single dose of the hamster antibody–diphtheria toxin conjugate prolonged the mean survival time of animals challenged concurrently with SV40-induced sarcoma cells and delayed the appearance of the tumour after transplantation. The conjugate, somewhat disappointingly, had no effect on established tumours of the SV40 fibrosarcoma but did cause regression of an SV40-induced lymphoma. The conjugate therefore seems to be more effective against the slow growing lymphoma than a rapidly proliferating tumour such as the fibrosarcoma, probably because of the appreciable delay in the entry of toxin molecules into cells and inhibition of protein synthesis after the initial binding to cell surface receptors. Faster growing tumours may replace damaged cells so successfully that overall growth is unimpaired.

The experimental design of these preliminary studies suffers from the use of whole toxin since the molecule, even when conjugated to antibody, still binds to cells through its own specific receptors. This undoubtedly contributes to the more or less severe secondary effects of therapy with toxin–antibody conjugate, such as damage to non-tumour cells and localised necrosis of subcutaneous tissue at the sites of injection. More positively, the toxicity of the toxin itself may be a useful adjuvant in the therapeutic action of toxin–antibody conjugates since this toxicity appears, to some extent, to be selective for tumours (Iglewski and Rittenberg, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2707–2710; 1974). Certainly, further effects along these lines would be worthwhile using perhaps a proteolytic C-terminal peptide fragment of diphtheria toxin that, although somewhat unstable, nevertheless carries almost the full toxic activity of the whole molecule when tested *in vitro* but cannot bind to surface receptors and therefore has no effect on intact cells lacking surface antigens recognised by anti-tumour antibody.

In another approach to a rational cancer therapy, R. Arnon and M. Sela and their colleagues (*Cancer Res.*, **35**, 1182–1186; 1975) have coupled an anti-tumour antibiotic, daunomycin, to antibodies directed against tumour-associated surface antigens. The difficulty is to prepare covalent complexes that preserve both the antibody activity

Core material at Earth's surface?

from Peter J. Smith

THE idea that the Earth's crust contains rock which once formed part of the upper mantle has become commonplace, especially since the work of Gass (*Nature*, **220**, 39; 1968) and others on the significance of ophiolites. Claims to have recognised material from the core, by contrast, are very much rarer. In view of the greater distances involved, the paucity of core derivatives at the Earth's surface is hardly surprising. On the other hand, modern concepts of mass flow in the mantle suggest that the transport of material from the core region to the crust should at least be possible; and it is to just such concepts that Bird and Weathers (*Earth planet. Sci Lett.*, **28**, 51; 1975) now appeal to explain the existence of josephinite along Josephine Creek in Oregon.

Josephinite is a rare rock containing a high proportion of nickel (55–75%) and iron (20–45%). In this respect it resembles, and has often been confused with awaruite, a somewhat less rare iron–nickel mineral associated with low-temperature reduction reactions occurring during the serpentinisation of ultramafic rocks. But a detailed petrographic and X-ray study of josephinite and awaruite carried out by Bird and Weathers shows that the two materials have significant differences. Chief among these is that josephinite contains lamellae and blebs of magnesium silicate and andradite garnet. Some of the least altered samples also contain elemental silicon and a natural equivalent of the artificial substance CaO.2"FeO", neither of which has previously been found in terrestrial rocks, least of all in awaruite.

The unique composition and structure of josephinite suggests to Bird and Weathers that, in contrast to awaruite, this material has a high-temperature and high-pressure origin, perhaps by crystallisation from an iron–nickel melt. This in turn suggests that the source could lie in the vicinity of the core–mantle boundary. And in the light of modern ideas, the most obvious way of transporting material from the core–mantle boundary to the upper lithosphere would be to have it carried upwards by mantle plumes and emplaced in the crust by means of associated diatremes. For as Bird and Weathers point out, "the distance of vertical transport from the core–mantle boundary region to the surface is certainly no more surprising than the lateral changes of distances established by oceanic anomaly patterns for the present plate population".

and the pharmacological activity of the anti-tumour drug. The most successful method developed by the Israeli group is periodate oxidation of the sugar moiety of daunomycin to generate aldehyde groups capable of forming Schiff base linkages to free amino groups on the antibody molecules. These linkages are then stabilised to secondary amines by sodium borohydride reduction. About 2 to 5 drug molecules could be substituted into each antibody molecule in a form with acceptable capacity for killing cells. The drug–antibody conjugates showed immunological specificity in the sense that conjugates containing antibody directed against different murine lymphoid tumours bearing specific surface antigens were cytotoxic only against the appropriate tumour or tumours bearing cross reacting surface antigens. It should eventually be possible to test *in vivo* the effectiveness of antibody-directed tumour cell killing by daunomycin, particularly as methods are improved for the preparation of tumour-specific antibodies. ☐

Nuclear shock waves

from P. E. Hodgson

THE concepts of classical physics can often be used to gain a useful insight into nuclear phenomena, especially when relatively large numbers of high energy nucleons are involved. An example of this is provided by what happens when two nuclei collide at high energy. In the region where they first interact the density rises suddenly, and if the relative velocity of the nuclei is greater than the rate of propagation of this density disturbance through the nucleus a nuclear shock wave develops.

The rate of propagation of the shock wave is the velocity of sound in nuclear matter and this can be estimated from the known nuclear compressibility (*Nature*, **254**, 480, 1975) to be that corresponding to an energy of about 10 MeV per nucleon for the colliding nuclei. Such energies can be attained by many heavy ion accelerators so it is possible in principle to produce nuclear shock wave phenomena.

The development of such shock waves has been calculated by Scheid, Mueller and Greiner (presented at Nashville Conference, 1974), and they find for ^{16}O–^{16}O collisions that even for centre-of-mass energies of 100 MeV densities almost twice normal appear For energies of 1,000 MeV, densities five times normal occur, and in each case a shock wave is expected.

The shock wave itself is essentially a sharp discontinuity in the nuclear pressure, density and velocity, and it is essential for its application to nuclei that the thickness of the shockwave front, or region of rapidly varying properties, is small compared with the nucleus itself. The thickness is approximately the same as the mean free path of the nucleons in the nucleus, and as this is rather less than 1 fm, whereas nuclei typically have diameters of a few fm, this condition is expected to be satisfied.

The shockwave phenomena occurring when two spheres of matter collide have already been worked out for stars, and have been applied to nuclei by Wong and Welton (*Phys. Lett.*, **49B**, 243; 1974). For simplicity they considered the collision of two slabs of nuclear matter in one dimension and used the Brueckner equation of state to calculate the density and energy of the nuclear matter in the shock wave as a function of the Mach number, the ratio of the velocity of the shock wave to that of the nuclear sound. They found that the shock wave propagates along the collision axis and that dissociation occurs at a Mach number of about two. Thus we expect to find showers of nucleons emitted in the forward and backward directions

If one of the colliding nuclei is much lighter than the other, the shock wave front is conical, with the semi-angle of the cone given by the ratio of the shock-wave and particle velocities, just like the electromagnetic wavefront in the Cerenkov effect. Figure 1 shows a schematic illustration of the development of such a shock wave when an ^{16}O ion collides with ^{238}U at energies of more than 10 MeV per nucleon. Such waves are emitted at a definite angle that can be related precisely to the energy of the incident particle If such an effect were observed in nuclei it could provide evidence of nuclear shock waves.

An attempt to detect nuclear shock waves in energetic heavy ion interactions has been made by the Berkeley group (Crawford *et al.*, *Phys. Rev. Lett.*, **34**, 329; 1975) by bombarding gold nuclei with 25 GeV ^{12}C ions. At such high energies, corresponding to velocities about a fifth of that of light, one might expect a nuclear shock wave to develop. They measured the angular distribution of fragments with charges from 5 to 9 and energies up to 1,000 MeV emitted from the nuclear disintegrations and looked for the peak around 50° that would be the definitive sign of a nuclear shock wave. The data did indeed show a slight maximum at this angle, but it was not statistically significant and cannot be interpreted as evidence for a shock wave.

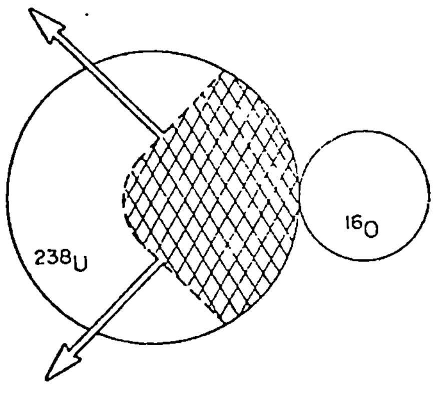

Fig. 1 Schematic illustration of the development of a shock wave front after the collision of an ^{16}O ion with a ^{238}U nucleus.

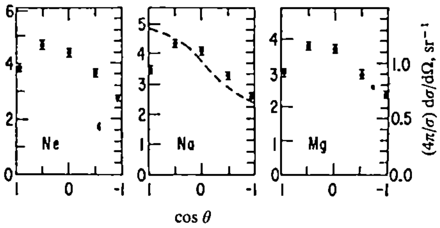

Fig. 2 Angular distribution of fragments from uranium irradiated by 28 GeV protons. The dashed curve refers to carbon emitted from uranium bombarded by 5.5 GeV protons, which shows no peaking.

Another attempt to detect nuclear shock waves has been made recently by Remsberg and Perry at Brookhaven National Laboratory (*Phys. Rev. Lett.*, **35**, 361; 1975). They irradiated gold and uranium with 28 GeV protons and looked at the energy spectra of the emitted fragments with charges between 6 and 12. As shown in Fig. 2, the spectra of some of the heavier fragments show a broad peak around 70°; for lighter fragments the peak shifts to smaller angles and becomes less distinct. These peaks occur at about the angles predicted for a nuclear shock wave, and may indicate the initiation of some kind of collective motion directly associated with the cascade of particles initiated by the incoming proton. More precise data would however be needed to establish the presence of a nuclear shock wave.

Another possible phenomenon that could occur in a nuclear shock wave is the production of what are called ultradense nuclei. These are stable, abnormally dense nuclei in which the energy of compression is compensated for by pion condensation. Such nuclei are at present purely theoretical conjectures, based on our present understanding of pion and nucleon forces. It is further conjectured that such nuclei might be formed in a nuclear shock wave, where the nuclear density may be raised two or more times above its normal value. This might be just enough to cause the nuclei, or perhaps fragments emitted from the energetic interactions, to condense to the ultradense state.

A search for ultradense nuclei has recently been carried out by another group at Berkeley (Price and Stevenson, *Phys. Rev. Lett.*, **34**, 409; 1975). They bombarded lead with relativistic argon ions having 1.1–1.6 GeV per nucleon, and looked for emitted particles of Z greater than 20 that might be interpreted as ultradense nuclei. No evidence for such particles was found.

There is thus no definite evidence at present either for nuclear shock waves or for ultradense nuclei, but they both provide a stimulus for more theoretical and experimental work, and show the continuing usefulness of classical ideas even in nuclear physics at high energies.

Managing Britain's forests

from Peter D. Moore

THE development of forestry in Great Britain is sheer economic good sense, or so the speakers at a recent conference on Forestry Management sponsored by the Royal Society of Arts in London would have us believe. Our current demand for wood and wood products in Britain is 0.84 m^3 per head of population and of this 0.76 m^3 is imported. Our total per capita forest resource amounts to only 0.03 ha, about a quarter of that enjoyed by, for example, West Germany. Of this resource, well over half is currently under private ownership and management. Undoubtedly world consumption of timber will continue to rise and our very considerable dependence upon imported timber will place us in a very vulnerable economic position. This fact alone seems ample justification for the expansion of our forestry, as an investment for the future.

The situation, however, is not simple. We live in a society in which the Chancellor of the Exchequer can pledge himself to the support of "a mixed economy in which the private sector is vigorous, alert and profitable", yet this Conference revealed severe discontent amongst the private sector of forestry concerning the proposed system of taxation. Under this, the owner of a forest is not only liable to pay income tax, but also may be liable to the proposed wealth tax and his timber, on harvesting presumably after his decease, will be subject to capital transfer tax. These represent serious disincentives to the potential private forester and evidently demand the close attention of those responsible for the creation of taxation systems. It is surely mindless cynicism, on the part of some, to have regarded afforestation as a technique for tax avoidance.

The British national forestry body, the Forestry Commission, has recently redefined its objectives, presumably out of deference to the vociferous environmental lobby in this country, so that it is no longer simply committed to the production of timber, but also to the "protection and enhancement of the environment and the provision of recreational facilities". One can forgive the foresters a degree of resentment regarding the insertion of these phrases since, as one speaker laconically remarked, no one has asked that the North Sea oil rigs should be painted in aesthetically pleasing colours. More people are likely to encounter forests than oil rigs, however, which places greater social responsibility upon the forester, but one must recognise that forest modification in the interests of amenity costs money or, more precisely, cuts profitability.

If forestry is to provide an amenity it may necessitate planting slower growing species and in such situations that overall productivity is reduced. The provision of amenity may also involve the Forestry Commission in permitting greater freedom of access to forests, which brings the attendant dangers of damage and fire. Undoubtedly one can also anticipate an increased demand for camping facilities, for hunting and angling and possibly for motor rallying. Not all such activities are compatible with one another; there are limitations to multipurpose uses of forest.

The relationship between British forest management and nature conservation is even more complex. It was recognised at the Conference that afforestation of upland moorland generally causes an increase in the diversity of wildlife inhabiting the area, especially during the early stages of forest growth. The replacement of deciduous by coniferous forest in lowland situations, however, has the reverse effect. Even in the uplands the maturation of forest usually brings about a final reduction in plant and animal diversity and many of the species of open moorland habitats will obviously be lost. What received little emphasis in the Conference was the justification for nature conservation. One received the impression that wild plants and animals, their study and conservation, belonged to the amenity of forest use. Although there is a measure of truth in this, it is far from the full story. Steele pointed out that many communities once destroyed could never be recreated, so the maintenance of these fragments of woodland which approximate to our native forest is particularly urgent if we are to have a yardstick against which to measure our achievements in the management of planted forests for timber production. Added to this there is that crucial argument for conservation based upon our limited genetic resources. The loss of a species of animal or plant, or even the loss of a local population of a species, involves genetic depletion. The consequences of any such loss cannot be calculated, but the repercussions could be economic as well as aesthetic, for one can never tell when a given species will assume economic importance to man.

From the point of view of nature conservation, the most efficient land use practice is the maintenance of habitat diversity alongside the afforestation programme. This attitude was admirably illustrated by the efforts of Lord Dulverton at his estate near Fort William, Scotland. He has adopted a mixed land use policy retaining moor-

A hundred years ago

THE Secretary of the Interior, in his annual report to the President of the United States, commends in high terms the work of the Geological and Geographical Survey of the Territories, and presents the following brief summary of the results for the season of 1875:— The survey under Dr Hayden continued its labours of the two preceding years in the Territory of Colorado . . . . . The districts explored in the past season were not so mountainous as those of the previous years, but were quite remote from settlements, and in perhaps the most inaccessible regions of this continent. The total area surveyed is about 30,000 square miles, portions of which were very rugged. Much of this area is drained by the Colorado river, and is mainly a plateau country cut in every direction by deep gorges or canons, the sides of which show, for geological investigations, admirable sections of the strata forming the earth's crust. The topography of the district surveyed was elaborated in detail by the aid of the plane-table. The exploration of the remarkable prehistoric ruins of Southern Colorado, glimpses of which were obtained the preceding season, was continued with great success. They were traced down the canons to the Colorado river in New Mexico, Utah, and Arizona, and their connection established with the cliff cities of the Moquis of the latter Territory. Hundreds of cave-dwellings, of curious architecture and many miles from water, were found in the sides of the gorges, and the ruins of extensive towns discovered in the adjacent plains, indicating the former existence of a people far more numerous and advanced in the arts of civilization than their supposed descendants of the present day. Of these ruins many interesting sketches, plans, and photographs were made, and a valuable collection of flint weapons, earthenware and other specimens, was gathered.

from *Nature*, **13** (December 23), 156; 1875.

land on upper slopes and combining forestry with improved pasture for sheep on the lower hills. Added to this he stressed the need for maintaining any existing areas of native deciduous woodland, as a genetic and aesthetic rather than an immediate economic investment. Such an attitude of compromise whether on the part of the Forestry Commission or private landowners would be welcomed by all conservationists. ☐

Plasticity of the nervous system

from Shin-Ho Chung

A Royal Society Meeting for Discussion, organised by H. B. Barlow and R. M. Gaze, was held on December 4 and 5 at the Royal Society, London.

FOR two days at the Royal Society, thirteen neurobiologists presented recent evidence dealing with the adaptive nature of the nervous system. Although the topics discussed ranged from the amphibian nerve–muscle preparation to the mammalian cortex, the problems posed by new discoveries converged on one central question: what are the underlying mechanisms that enable synapses in the nervous system to be changed in response to an altered external environment? This turns out to be an enormously difficult question to answer.

The simplest system in which plasticity of synaptic connections can be demonstrated is the nerve–muscle junction. It has been known for many years that muscle fibres, when deprived of their original nerves, accept newly formed branches of foreign nerves with which they do not normally connect, but the fate of such aberrant synapses when the cut nerve regenerates is under dispute. R. F. Mark (Australian National University) presented new evidence supporting his earlier claim that 'incorrect' synapses are suppressed when 'correct' synapses are reformed, rather than both remaining active. He suggests that nerve fibres compete for the available synaptic sites, and, if two nerves innervate one target cell, the less preferred one is rendered inactive. Because such a mechanism may account for several puzzling facts noted in the central nervous system, Mark's observations need to be carefully reexamined. The phenomenon of collateral sprouting, as described above, is also known to occur in the mammalian brain. G Raisman (National Institute for Medical Research, Mill Hill) reported that vacated synaptic sites in the septal nucleus and in the superior cervical ganglion can be re-occupied by other nerves. When the vagal or hypoglossal nerve is directed to a denervated ganglion, vacated synaptic sites are occupied by the new nerve, each fibre subserving about the same number of sites as the original one.

Raisman's observations clearly demonstrate that specificity for afferent–efferent coupling in the nervous system is not rigid, and under certain conditions a foreign axon is as competent in occupying synaptic sites as the original one. If synaptic coupling between an axon and a target cell is not rigidly stipulated, it is conceivable that an axon may form a large number of aberrant contacts in the course of embryonic development. P. D. Wall (University College London) argued that even in the adult, axons have a far wider anatomical distribution than post-synaptic physiology would suggest. Wall provided evidence that an axon entering the cat spinal cord traverses and forms synaptic contacts over several spinal segments. The distant connections are normally suppressed, but their presence can be unmasked by stimulating the axon electrically. The mechanisms by which such synapses are suppressed remain unknown, as are the means by which post-synaptic cells recognise appropriate axons. There are known to be several regions in the nervous system where an axon forms a series of transient connections during development before establishing stable contacts with post-synaptic cells. M. J. Keating (National Institute for Medical Research, Mill Hill) adduced that the fibres connecting the two optic tecta in *Xenopus* shift their connections continually during development. Such re-adjustments are necessitated by the gradual movement of the eyes relative to the animal's head; in order to register an image seen by the two eyes onto the same area in the tectum, a series of synaptic contacts are temporarily formed and subsequently broken. He presented elegant evidence that the ability of a nerve fibre to reform new connections declines slowly with age. This discovery may be a neurophysiological basis for human learning.

A large part of the meeting was devoted to the mammalian visual system. Anatomical studies have shown that optic axons terminate in bands of cells in the geniculate body, fibres from each eye ending in alternating layers. All the axons originating from the geniculate body project onto the fourth cell layer of the visual cortex, where alternating clusters of fibres subserving one eye are interdigitated with those of the other, giving the appearance of a chequerboard pattern. From his spectacular series of anatomical studies. P. Rakic (Harvard University) elucidated the sequence of events leading to the formation of these intricate anatomical arrangements in the rhesus monkey: Cells in the different visual centres divide mitotically at different embryonic stages; those forming the geniculate body, for example, are all generated within a few days, whereas the cortical cells are continually being formed over a period of two months. Before the geniculate cells are segregated into several distinct layers, optic nerve fibres invade the region, those from one eye intermingling with those from the other. Similarly, fibres receiving the inputs from the left and right eyes do not form regularly spaced clusters but are distributed as a continuous sheet in the visual cortex. From such amorphous masses of cells and fibres, the ordered patterns of fibres carrying inputs from the right and left eyes gradually evolve.

Because visual fibres from both eyes project to the cortex, most post-synaptic visual cells respond to stimulation of either eye. When an animal is reared with one eye closed, cortical cells then respond predominantly to the eye which has been left open. C. Blakemore (University of Cambridge) showed that in the kitten only a brief period of sensory deprivation during the critical stages causes all cortical cells to be heavily dominated by the normal eye. Evidence that such an occular dominance, once patterned, is not modifiable by extensive retraining of the deprived eye later in the animal's life was provided by K. -P. Hoffman (University of Mainz). S. LeVay (Harvard University) demonstrated the anatomical basis of occular dominance in a rhesus monkey deprived of vision in one eye for a year. The alternating clusters of terminals from the right and left eyes in the cortex are no longer of equal size but instead clusters from the normal eye gradually expand, and those from the deprived eye shrink.

There may indeed be a common basis for all the diverse facts put forward at this meeting. If we can unravel the underlying mechanisms by which the visual centres become ordered so exquisitely, we will then understand how terminals there expand and shrink as a result of monocular visual deprivation. Similarly, if the means by which nerve cells recognise synaptic contacts as appropriate or inappropriate are elucidated, it will also become clear how nerve cells make continual adjustments and re-adjustments of their synaptic relations during development. These are problems for which neurobiologists cannot even begin to formulate questions. Answers will be difficult to find when one does not even know what questions to ask. ☐

articles

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Chlorophyll arrangement in a bacteriochlorophyll protein from *Chlorobium limicola*

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The three-dimensional structure of a chlorophyll-containing protein has been determined by X-ray crystallography and shown to consist of three identical subunits, each containing a core of seven bacteriochlorophylls arranged in an irregular fashion and enclosed within an envelope of protein.

The three-dimensional structure of a bacteriochlorophyll protein has been determined by X-ray crystallography. This is the first chlorophyll-containing protein for which the structure is known, and provides new information on the arrangement of chlorophyll *in vivo*. The bacteriochlorophyll protein is seen to consist of three identical subunits, each containing a core of seven bacteriochlorophyll molecules arranged in an irregular fashion and enclosed within an envelope of protein.

Green photosynthetic bacteria contain small amounts of a water-soluble bacteriochlorophyll protein which can be extracted in aqueous salt solutions without the use of detergents[1,2]. This complex has been shown to mediate in the transfer of excitation energy from the antenna pigment chlorobium chlorophyll to the photochemical reaction centres of the organism[2–4]. The complex from *Chlorobium limicola* has been shown by chemical studies to have a molecular weight of about 150,000 and to contain 21 ± 2 molecules of bacteriochlorophyll *a* (Bchl *a*), tightly, though not covalently associated with the protein[5]. Analyses of the absorption and circular dichroism spectra, recorded at 77 K, gave evidence for interaction between at least five molecules of Bchl *a* (ref. 6). Preliminary X-ray studies of two different crystal forms of the Bchl protein have shown it to be a trimer consisting of three identical subunits[7]. The form in space group $P6_3$ with unit cell dimensions: $a=b=112.4$ Å and $c = 98.4$ Å, has one subunit per asymmetric unit and was used for the X-ray structural studies reported here. In this paper we describe the arrangement of the Bchl *a* molecules within the complex as deduced from a 2.8-Å resolution electron density map. Description of the X-ray structure determination will be confined to essential features and a comprehensive analysis will be presented elsewhere.

Structure determination

Details of the procedure used for growing large crystals of the Bchl protein have previously been described[7,8]. Diffraction data from the native crystals and isomorphous heavy atom derivatives were recorded photographically using an Elliott rotating anode X-ray generator and Enraf–Nonius precession cameras. Integrated intensities were measured from conventional precession photographs by means of a computer controlled Optronics rotating drum microdensitometer[9]. After symmetry averaging and merging the intensities from 23 different film packs, each set of data reduced to about 16,500 unique reflections, excluding the Friedel related measurements. This total includes 90% of the data to Bragg spacings of 2.8 Å together with 42% between 2.8 Å and 2.75 Å spacings. Excluding the weakest reflections, the mean error in amplitude on scaling together planes within the native and derivative data sets ranged from 2.8 to 3.4%. The crystals contain about 70% by volume solvent, and presumably for this reason do not diffract strongly, particularly at high angles. The resolution of 2.8 Å adopted for this study is the highest which could reasonably be attained using Buerger precession photographs.

Isomorphous heavy atom derivatives were prepared by soaking crystals of the native protein in solutions of heavy atom compounds in 0.01 M Tris-HCl buffer containing 1 M NaCl and 10% w/v $(NH_4)_2SO_4$ at *p*H 7.8. Two suitable derivatives were obtained by soaking in the presence of 5×10^{-4} M potassium chloroplatinite and saturating concentrations of methyl mercuric iodide, each for 7 d. A third, double derivative, was made by soaking in a solution containing both 5×10^{-4} M potassium chloroplatinite and an excess of methyl mercuric iodide, again for a period of 7 d. One heavy atom site in each of the two single derivatives was initially located from a difference Patterson synthesis of the hk0 zone. Additional sites in the mercury and double derivatives were then found from projection and three-dimensional difference Fourier maps in the usual way. Further refinement of the heavy atom parameters was carried out using a lack-of-closure least squares refinement procedure applied to a subset of the three-dimensional data, selected to include all of the centric data together with 25% of the acentric reflections[10]. The heavy atom parameters used in the final phase calculation are listed in Table 1.

"Best" phases were calculated by the multiple isomorphous replacement method including contributions from measurements of anomalous scattering of the heavy atoms[11–13] The mean figure of merit of 0.57 is probably a conservative estimate since the value of 0.66 for the average r.m s. lack of closure over E indicates that the E values used had been overestimated A comparison of the mean heavy atom scattering with the E values (Table 1) shows that the single site platinum derivative contributes substantially less to the phasing than do the mercury and double derivatives. This is not surprising in view of the difficulty in measuring the comparatively small changes in intensities produced by the introduction of a single platinum atom into an asymmetric unit of this size. The Fourier calculation was performed using a fast Fourier transform

Table 1 Heavy atom parameters

Derivative	Z	x	y	z	B	N	$\langle f_h \rangle$	E	R (%)
Potassium chloroplatinite	28.2	0.113	0.443	−0.251	27.5	5,445	44	35	52
Methyl mercuric iodide	40.3	0.379	0.582	−0.007	25.1	5,444	75	38	47
	16.7	0.301	0.439	−0.104	34.6				
	9.3	0.279	0.434	−0.143	9.9				
Double derivative	31.4	0.113	0.443	−0.250	35.0	5,460	85	43	52
	38.7	0.380	0.582	−0.006	25.9				
	14.1	0 301	0.437	−0.104	32.8				
	10.0	0.278	0.434	−0.142	16.4				
	9.6	0.510	0.204	−0.397	23.8				

Z, relative occupancy of heavy atom site in arbitrary units; x, y, z, fractional coordinates; B, isotropic temperature factor (Å^2); N, number of reflections included in the refinement. $\langle f_h \rangle$, E and R are respectively the mean heavy atom scattering, the lack of closure error and the residual for the centric reflections to a resolution of 2.8 Å.

program provided by L. F. Ten Eyck[14]. The electron density distribution was sampled at intervals of 0.73 Å (c/135) normal to the c axis and the contour levels traced on to Dayco plastic sheets stretched on to aluminium frames, suitable for observation in an optical comparator[15,16].

Description of the molecule

We have suggested previously that in the P6$_3$ crystal form the trimers are positioned on the threefold rather than the 6$_3$ axes of the unit cell[7]. The electron density map clearly confirms this conclusion. The packing arrangement gives rise to extensive channels of solvent 112.4 Å (the a cell dimension) apart and approximately 62 Å in diameter centred on the 6$_3$ axes and running parallel to the c axis throughout the crystal. Each trimer extends 57 Å along the threefold axis and has a maximum diameter of 83 Å at right angles to it. Thus, the overall dimensions of the trimer correspond closely to those deduced earlier from considerations of symmetry and molecular packing. It was possible to define the inter-subunit boundary by making only one cut through contoured electron density. The subunits are, however, packed very closely together and extensive interactions between them undoubtedly occur at the interfaces. In contrast, the contacts between one trimer and its neighbours in this crystal form are very tenuous.

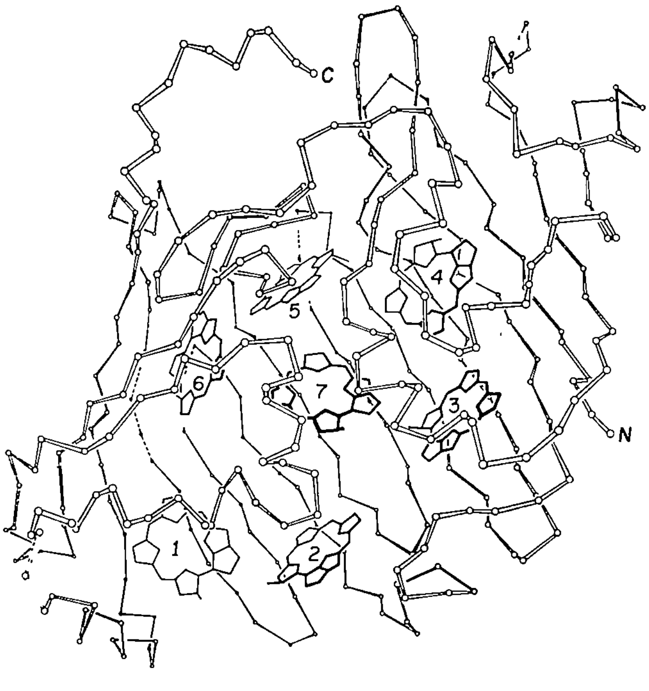

Fig. 1 Schematic diagram showing the arrangement of the polypeptide backbone and chlorophyll core of one subunit of the bacteriochlorophyll protein. Presumed α-carbon positions are indicated by circles. The connectivity of the polypeptide chain is in some instances uncertain (see text), and particularly ambiguous regions are indicated by broken lines. For clarity, the magnesium atoms, the chlorophyll ring substituents and the phytyl chains, except for the first bond, are omitted. The direction of view is from the threefold axis, which is horizontal, towards the exterior of the molecule.

The amino acid sequence of the Bchl protein has not yet been determined, it was therefore necessary to rely solely on the continuity of the electron density in attempting to determine the course of the polypeptide chain. By placing markers in the electron density map at probable α-carbon positions it was possible to follow extensive sections of the protein backbone. In some regions, however, particularly in loops on the surface of the molecule, breaks in the continuity do occur and at this stage we are unable to define a unique course for the whole of the polypeptide chain. But, apart from a few ambiguities, the continuity is generally quite good, and is sufficiently defined to give an overall view of the protein conformation. The total number of amino acid residues in each subunit, based on the number of marked α-carbon positions is 327, although it is probable that some residues have been missed in regions where the electron density is not well defined. Assuming a mean residue weight of 110, calculated from the amino acid composition[5], the minimum molecular weight of each subunit, including Bchl, becomes 42,300, and for the trimer 127,000.

The conformation of the polypeptide chain is illustrated in Figs 1 and 4, and can be seen to form a distorted hollow cylinder. In our present interpretation of the electron density map, one end of the cylinder seems to be "open", but is occluded by the carboxyl-terminal helix of an adjacent subunit of the trimer. It is also possible to interpret the electron density map in the vicinity of this inter-subunit contact in such a way that a single subunit would form a "closed" hollow cylinder. At present we cannot distinguish with certainty between these two possibilities. The hollow cavity within the cylinder contains the Bchl aggregate. The area of the cylindrical wall which is exposed to the solvent in the trimer is composed almost entirely of 15 strands of β sheet which seem to be mainly anti-parallel. The side of the cylinder in contact with an adjacent subunit has a more complex structure, consisting of four short lengths of α helix interspersed with regions of irregular conformation

Bacteriochlorophyll core

Within the core of each subunit the electron density clearly corresponds to an array of chlorophyll molecules. Disks of density indicate the positions of the porphine rings, whereas the phytyl side chains can be seen as resolved ribbons of density. Seven Bchl molecules pack within one subunit, corresponding to a total of twenty-one for the trimer, in agreement with the chemical studies[5]. The chemical structure of Bchl a is known (Fig. 2), and differs from chlorophyll a in having two extra hydrogen atoms in ring II and an acetyl group in place of the vinyl group on ring I. The absolute configurations at the asymmetric carbon atoms in rings II, IV and V have been established[17,18]. In addition the X-ray crystal structure of ethyl chlorophyllide *a* (a *trans*-esterified derivative of chlorophyll *a* in which the phytyl group is replaced by ethyl) has been determined[19–21].

The seven Bchl *a* molecules are confined within an ellipsoid of axial dimensions: 45×35×15 Å (Fig. 3). The average centre–centre nearest neighbour distance between porphine rings is 12 Å, which may be compared with 24 Å, the closest distance between chromophores in adjacent subunits. The orientations of the porphine rings do not conform to any repetitive pattern, although their planes do, to a first approximation, lie parallel with one another. This may be demonstrated by taking the mean of the direction cosines of the perpendiculars to each of the rings to define a plane which is most nearly parallel with the planes of the seven porphine rings. The angles made by the individual porphine planes with this calculated plane lie between 10° and 40°. In contrast, the azimuthal orientations of the near coplanar rings vary considerably. The normal to the calculated plane makes an angle of 62° with the threefold axis of the trimer and the centre of the aggregate in each subunit is at a distance of about 20 Å from the threefold axis. Thus, in the complete trimer the Bchl *a* aggregates of the individual subunits combine to form a triangular funnel about the threefold axis.

In each subunit the phytyl chains lie close together, sandwiched between five of the porphine rings on one side and the other two rings and the β sheet wall of protein on the other. The hydrocarbon chains are in most cases in an extended conformation, although in one instance (Bchl 1) the chain is bent into a complete U-shaped loop. The phytyl chains of chlorophylls 4, 5 and 6 lie parallel, forming a planar structure in close contact with the β-sheet wall of the protein. This hydrophobic core of phytyl chains is completely buried in the protein, a thermodynamically favourable arrangement analogous to the interior of other globular proteins in which most of the hydrophobic residues are found buried out of contact with the solvent. A characteristic of β structure is the alternation of the directions of the amino acid side chains relative to the plane of the sheet. It seems likely that in this case the inwardly directed side chains are predominantly hydrophobic, forming close interactions with the phytol aggregate, whereas the residues directed towards the exterior are largely hydrophilic, forming favourable interactions with the solvent. The β sheet thus provides a suitable amphipathic layer, shielding the hydrophobic Bchl aggregate from the aqueous surroundings. Since the direction of the side chains alternates along the polypeptide chain, it will be interesting to see whether the sequence of these strands conforms to a pattern of alternating hydrophobic and hydrophilic residues

The electron density map gives no evidence for chemical interactions between neighbouring chlorophyll molecules, either direct or through bridging water molecules, other than the hydrophobic associations which undoubtedly occur between the phytyl chains There is, however, good evidence for extensive interactions between the chlorophyll molecules and the surrounding protein shell. The presence of a fifth ligand to the Mg atom of each of the Bchl *a* molecules is indicated by electron density protruding from the centres of each of the porphine rings. In five cases this density extends to form a continuous bridge between the Mg atom and the polypeptide chain. Without knowledge of the amino acid sequence it is not possible at this stage to characterise these ligands. Peptide oxygens or amino acid side chains such as Asp, Glu and their amides, Ser, Thr, His or Met could interact with the Mg directly or by hydrogen bonding to a coordinated water molecule. In addition, the Bchl *a* ring substituents include a number of potential hydrogen bond acceptors such as the oxygen-containing groups mentioned earlier. Most of these groups are close to the periphery of the aggregate and are sufficiently close to the protein electron density to be expected to form hydrogen bonds with suitable donors in the polypeptide chain. Thus, it seems that the Bchl *a* molecules are anchored to the protein through extensive hydrogen bonding and liganding to the Mg atom, in addition to hydrophobic interactions. The distribution and orientations of the chlorophyll molecules are therefore governed largely by specific interactions with the protein rather than with one another. The Bchl molecules do not occur in regular one- or two-dimensional arrays as has been observed in crystal structures of chlorophyll derivatives, and postulated as models for chlorophyll arrangement *in vivo*[19–22].

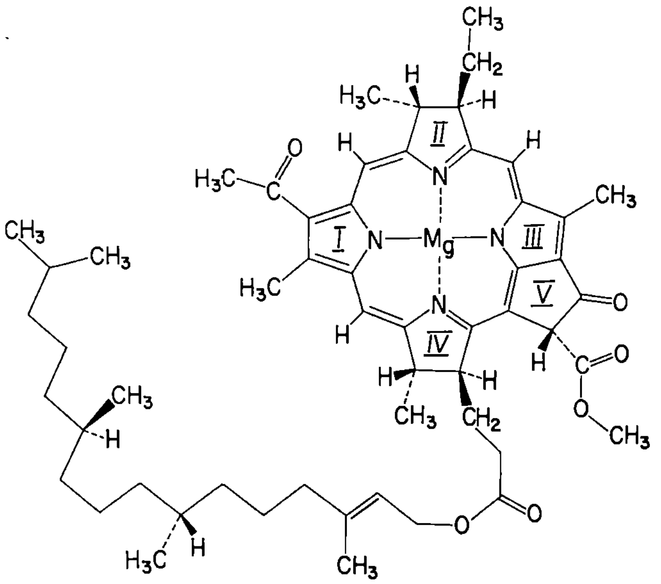

Fig. 2 Structure of bacteriochlorophyll *a*, indicating the absolute configurations of the asymmetric carbons atoms.

Energy transfer

Fluorescence studies suggest that in the green photosynthetic bacteria this Bchl *a* protein functions as an intermediary in the transfer of excitation energy from the antenna pigment, chlorobium chlorophyll 660, to the photochemical reaction centre, P840. Mechanisms for energy transfer between the components of the photosynthetic apparatus of both bacteria and higher plants have recently been discussed by several authors[23–25]. Energy transfer between chromophores separated by relatively short distances (10–20 Å) may be described in terms of an exciton model[26–28]. For a given electronic transition, coulombic interactions between the transition dipole moments of neighbouring identical, or near identical, molecules in an aggregate give rise to a set of *N* discrete exciton energy levels, where *N* is the minimum number of interacting species. Splitting of the main 809-nm absorption band and the presence of a complex CD spectrum at liquid nitrogen temperatures have been observed for this Bchl *a* protein. These observations were interpreted in terms of an exciton interaction involving a minimum of five or possibly six Bchl *a* molecules with estimated nearest neighbour distances of 12–15 Å (ref. 6 and J M. Olson, unpublished). The work described here shows that there are in fact seven Bchl *a* molecules separated by average nearest neighbour distances of 12 Å in each subunit of the trimer. The interactions between Bchl *a* molecules within each subunit thus appear to be appropriately described in terms of an exciton model. The magnitudes of the exciton interactions are critically dependent upon the relative orientations of the transition dipoles and on the inverse cube of the distances between the chromophores. We have observed a high degree of non-uniformity in the azimuthal orientations of neighbouring Bchl molecules in this aggregate (Fig. 3). This observation is in good agreement with an interpretation of the complex low temperature absorption and CD spectra in terms of the exciton theory. Furthermore, since the positions and orientations of the Bchl *a* molecules are now known, it should be possible to calculate the expected exciton interactions, and hence estimate the numbers and magnitudes of the absorption splittings and components of the CD spectrum. Such calculations for model systems using experimentally determined dipole moments or calculated point monopoles have been described[29,30]. It is hoped that this Bchl *a* protein will provide a useful model for testing current theories of exciton interactions between chlorophyll molecules; so enabling more reliable structural evaluations to be made for other chlorophyll containing systems for which multicomponent CD spectra have been observed[31-32].

In comparison with the Bchl protein described here, very little is known of the environments of the light-harvesting chlorobium chlorophyll 660 and the reaction centre, P840; and virtually nothing about the relative separation and spatial distributions of all three components[33]. Any discussion of the mechanism of energy transfer to and from the Bchl *a* protein remains speculative in the absence of such information. At chromophore separations of 50–100 Å, energy transfer is thought to occur by resonance, which, unlike exciton transfer, has an inverse sixth power dependence on separation distances[34]. If, however, the other chlorophyll components occur in proteins with molecular dimensions comparable with the Bchl *a* protein, and these proteins are closely associated in the membrane, then energy transfer would be required to occur over distances in the range of 20–50 Å (as for example between subunits of the Bchl-protein trimer) intermediary between the exciton theory and Forster's resonance transfer.

Chlorophyll proteins

The results described here represent the first structure determination of a chlorophyll-containing protein. In common with other globular proteins it contains the usual types of secondary structure such as α helices and β sheet. Unlike any other protein for which the X-ray crystal structure has been determined, however, it contains a large non-protein core as was originally proposed by J. M. Olson[35]. Presumably both the protein and the Bchl are necessary to maintain the integrity of the complex. The molecule is also unusual in being trimeric, having a cyclic arrangement of subunits involving extensive heterologous interactions. The functional significance of the trimeric nature of the molecule is obscure, though if the protein were extrinsically associated with the photosynthetic membrane it could indicate the presence of an intrinsic membrane component also possessing a threefold axis of symmetry.

Recent progress in the isolation and partial characterisation of a number of reaction centre and antenna chlorophyll proteins from photosynthetic membranes suggests that most, if not all, of the chlorophyll in both green plants and bacteria occurs in close association with relatively low molecular weight proteins[36,37]. The Bchl *a* protein described here is somewhat atypical in that it is highly water-soluble, whereas most other chrorophyll-proteins require the use of detergents to facilitate their solubilisation from membranes. There is however no evidence at present to suggest that the organisation of chlorophyll in these hydrophobic proteins should be grossly different from the model we have described. All of the current models describing the state of chlorophyll *in vivo* place emphasis on either direct

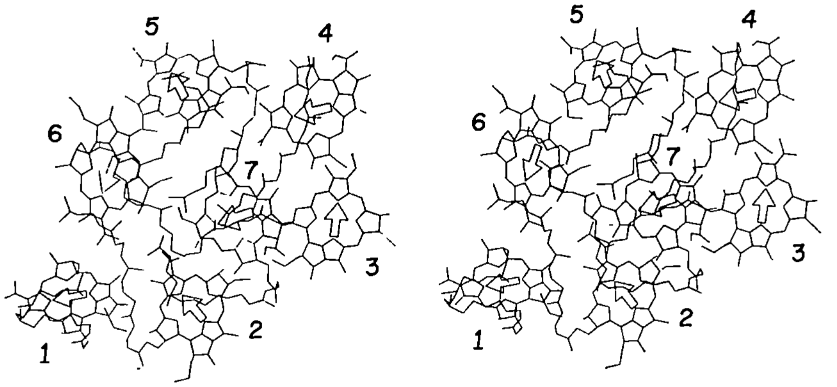

Fig. 3 Stereo diagram illustrating the chlorophyll arrangement within one subunit of the bacteriochlorophyll protein. The direction of view is approximately normal to the "average" chlorophyll plane (see text). The arrows, directed from ring III to ring I, are parallel to the Q_y transition moments of the respective bacteriochlorophylls. The magnesium atoms are omitted.

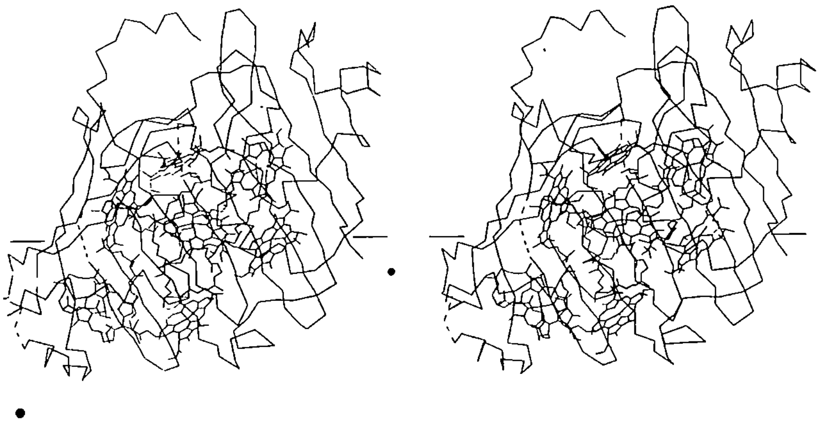

Fig. 4 Stereo drawing showing the arrangement of the chlorophyll core within the polypeptide envelope of the bacteriochlorophyll protein. The direction of view is the same as in Fig 1, and the location of the threefold axis is indicated by the two horizontal lines.

interaction involving Mg liganding or hydrogen bonding via bridging water molecules as the major factors determining the relative orientations of the porphine rings[19,38,39]. Our new findings for this particular Bchl-protein indicate that interactions between chlorophyll and protein including liganding to the magnesium atom, hydrogen bonding and hydrophobic interactions are of major importance in determining the arrangement of the chlorophyll molecules. Furthermore we suggest that these types of interaction could be of universal significance in determining the state of chlorophyll *in vivo*

Our results may be summarised as follows: the three-dimensional structure of a chlorophyll-containing protein has been determined by X-ray crystallography and shown to consist of three identical subunits, each containing a core of seven bacteriochlorophylls enclosed within an envelope of protein. The bacteriochlorophyll molecules are confined within a flattened disk-shaped region with their porphine rings lying roughly parallel to the disk. In contrast to current models for chlorophyll arrangement *in vivo*, the chlorophyll packing is dominated by interactions between chlorophyll and protein rather than between chlorophyll and lipid or between adjacent chlorophyll molecules. Furthermore the chlorophylls are arranged in an irregular fashion rather than in strictly ordered one-dimensional or two-dimensional arrays. It is suggested that the arrangement of chlorophyll seen here, in close association with protein, typifies the usual arrangement of chlorophyll *in vivo*.

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Identification of two related pentapeptides from the brain with potent opiate agonist activity

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Enkephalin, a natural ligand for opiate receptors is composed of the pentapeptides H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH. The evidence is based on the determination of the amino acid sequence of natural enkephalin by the dansyl-Edman procedure and by mass spectrometry followed by synthesis and comparison of the natural and synthetic peptides.

TERENIUS and Wahlström[1,2] and Hughes[3] have described the existence of an endogenous substance in the brain which acts as an agonist at opiate receptor sites. We later characterised this substance, termed enkephalin, as a low molecular weight peptide[4]. Other workers[5] have also confirmed the presence of a substance in the brain that competes for opiate binding sites and this substance, although not completely characterised, seems similar to enkephalin. A further peptide with opiate agonist actions, larger and chemically dissimilar to enkephalin, has been discovered in the pituitary gland[6]. We have now found that enkephalin is composed of two pentapeptides which we have identified and synthesised.

Enkephalin was isolated from pig brains as previously

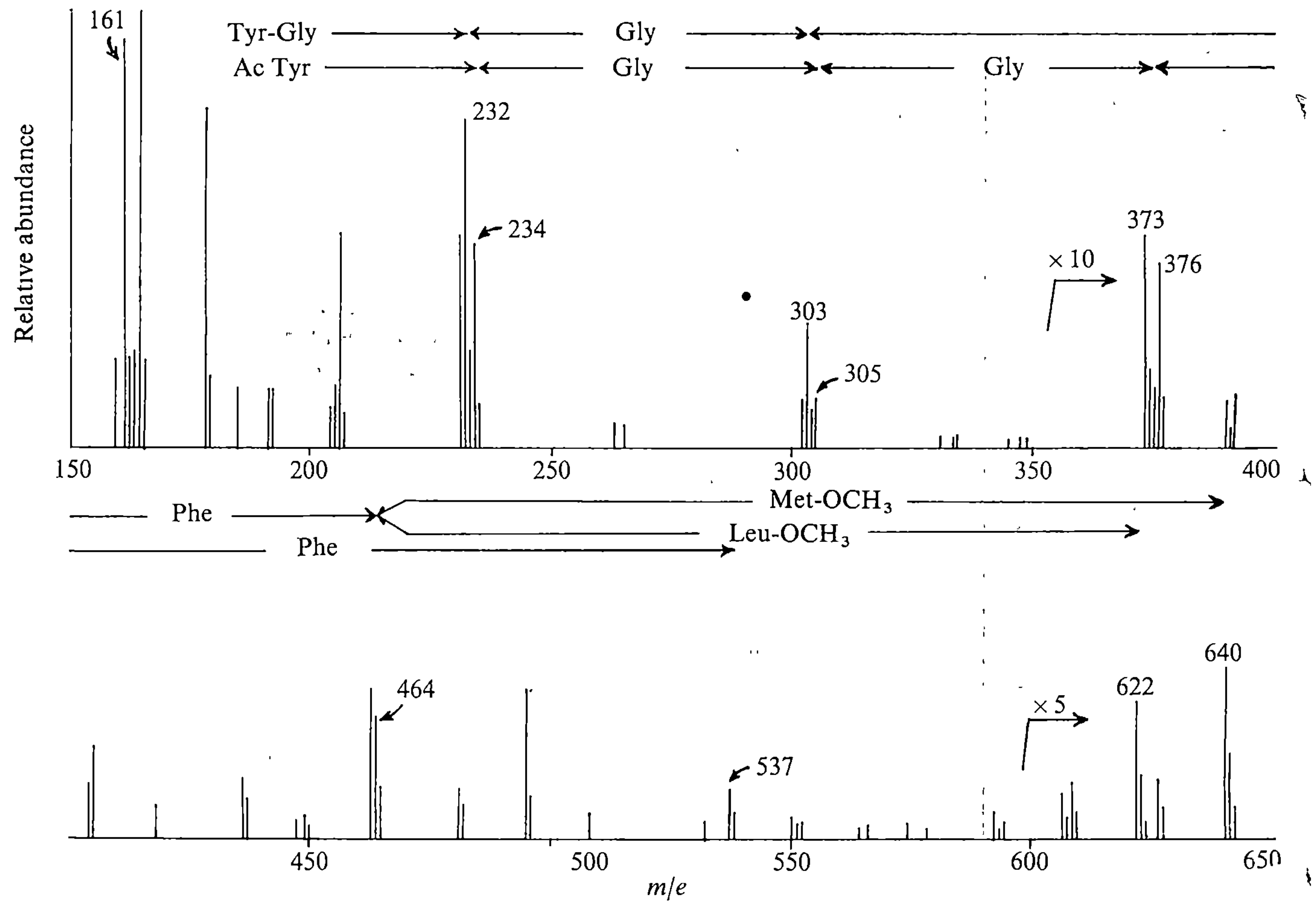

Fig. 1 Mass spectrum (above *m/e* 150) of the acetyl permethyl derivative of natural enkephalin. The assignment of the two pentapeptide sequences shown was based mainly on the recognition of an N–C cleavage pathway[10]; other modes of fragmentation, consistent with these structures, are present in the spectrum and will be described elsewhere. The spectrum also shows that enkephalin does not contain an unusual amino acid[4].

described[3,4]. The amino acid composition after hydrolysis with 5.5 N HCl at 110 °C for 20 h was: glycine 36.5, methionine 12.3, tyrosine 16 9, phenylalanine 22.5 and leucine 4.2 nmol. Tryptophan was not detected after hydrolysis with 3 N mercaptoethanesulphonic acid[7] at 110 °C for 65 h.

The amino acid sequence was first investigated by sequential degradation using the dansyl–Edman procedure[8]. This indicated the sequence Tyr–Gly–Gly–Phe– for the first four residues. Two amino acids were tentatively assigned to the fifth position, one clearly corresponding to methionine sulphone and another corresponding to leucine but giving a weaker spot. No further dansyl derivatives were detected after position 5 The chain length of the peptide could not be definitely fixed either by amino acid analysis or by the dansyl–Edman procedure. Molecular weight determinations by electrophoretic mobility[4] indicated a value of approximately 800.

The resulting uncertainties about the chain length and sequence assignment at position 5 were resolved by independent mass spectrometric analysis. The mass spectrum of the N-acetyl permethyl derivative[9] of natural enkephalin is shown in Fig. 1. It is interpreted as arising mainly from a pentapeptide sequence H–Tyr–Gly–Gly–Phe–Met–OH where methionine is C terminal This conclusion is substantiated by the results of deuterium labelling (*m/e* 640 shifts to *m/e* 658 on treatment with CD_3I (ref. 11)) and examination of enkephalin treated with cyanogen bromide (*m/e* 640 shifts to *m/e* 624). The presence of the *m/e* 622 signal is not entirely consistent with this interpretation. It seems to indicate the presence of H–Tyr–Gly–Gly–Phe–Leu–OH having a C-terminal leucine; alternatively it could be due to the loss of C_7H_7 from a putative molecular ion at *m/e* 713 for the methionine peptide.

The peptide sequences thus assigned to natural enkephalin from the mass spectra were synthesised by classical solution methods, the details of which will be published elsewhere. The mass spectrum of synthetic H–Tyr–Gly–Gly–Phe–Met–OH as the N-acetyl permethyl derivative has been found to be identical with that of natural enkephalin with the major exception that *m/e* 622 is absent in the synthetic material. On the other hand, the mass spectrum of the N-acetyl permethyl derivative of synthetic H–Tyr–Gly–Gly–Phe–Leu–OH differs from that of the natural enkephalin mainly by the absence of *m/e* 640 and the presence of *m/e* 622 as the most abundant high mass signals. The spectrum of the derivative of a mixture of the two synthetic peptides is identical with that of natural enkephalin. The mass spectrum of natural enkephalin is therefore interpreted as being the result of a mixture of the two pentapeptides H–Tyr–Gly–Gly–Phe–Met–OH (methionine-enkephalin) and H–Tyr–Gly–Gly–Phe–Leu–OH (leucine-enkephalin). It is estimated that the enkephalin isolated by us has a ratio of methionine-enkephalin to leucine-enkephalin of 3 or 4 to 1. This conclusion is in agreement with the results of the amino acid analysis.

The electrophoretic mobilities of the natural enkephalin and of the synthetic methionine- and leucine-enkephalins were determined at *p*H 2 and 2.5 kV on Whatman 3MM paper using 1-diaminonaphthalene-5-sulphonic acid as a neutral marker. A mobility of +0.61 relative to valine was found for natural enkephalin and its synthetic constituents. Mixtures of natural enkephalin with the synthetic peptides migrated as one spot.

The detailed pharmacology of the synthetic peptides will be presented in a later paper. Both methionine- and leucine-enkephalins have potent agonist activity at opiate receptor sites in that they produce a dose-related inhibition of electrically evoked contractions of the mouse vas deferens[12] and the guinea pig ileum[13] (Fig. 2). These inhibitory effects could be completely antagonised by naloxone (Fig. 3). Methionine-enkephalin is twenty times more active than normorphine in the vas deferens and equipotent with normorphine in the guinea pig ileum (Fig. 2). Preliminary results on the inhibitory effects of methionine-enkephalin on the stereospecific binding of ^{3}H-naloxone in Na$^+$-free homogenates of guinea pig brain indicate that methionine-enkephalin is at least three times more potent than morphine. Leucine-enkephalin has half the potency of that of methionine-enkephalin in the vas deferens but in the guinea pig ileum only one-fifth of that of methionine-enkephalin.

Natural enkephalin has been found to be 5–10 times more active in the vas deferens than in the guinea pig ileum[3], and the potency of natural enkephalin in the vas deferens, relative to normorphine, was found to be 15 to 1 in this study where the amount of enkephalin was determined by amino acid analysis. There is thus a very close agreement between the pharmacological activities of the natural and synthetic peptides.

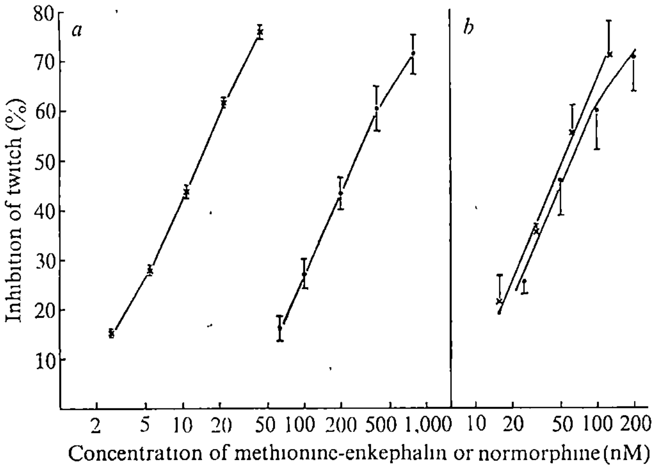

Fig. 2 Dose–response curves for normorphine (●) and synthetic methionine-enkephalin (×) in the mouse vas deferens (*a*) and guinea pig ileum (*b*). Potency ratios, enkephalin/normorphine: 21.5±4.5 (*n* = 4) in the mouse vas deferens and 1.29±0.35 (*n* = 3) in the guinea pig ileum.

In view of the correspondence between the natural and synthetic peptides in terms of their biological potencies, their mass spectra and electrophoretic mobilities, we conclude that our assigned structures for the two peptides comprising natural enkephalin are correct. At present, it cannot be decided whether the morphine-like peptides of Terenius and Wahlström[1] and Pasternak *et al.*[5] are identical with the natural enkephalin characterised by us. It is particularly interesting that the methionine-enkephalin sequence is present as residues 61 to 65 in β-lipotropin isolated from pituitary glands of sheep[14], pig[15] and man[16]. Attention has already been drawn[14–16] to structural relationships between β-lipotropin and β-MSH and ACTH. It is tempting to speculate that a similar relationship may exist between β-lipotropin and the peptides with opiate agonist activity obtained by Goldstein and colleagues from bovine pituitary[17] and crude ACTH[18] and that β-lipotropin may be a common precursor for these peptides and methionine-enkephalin.

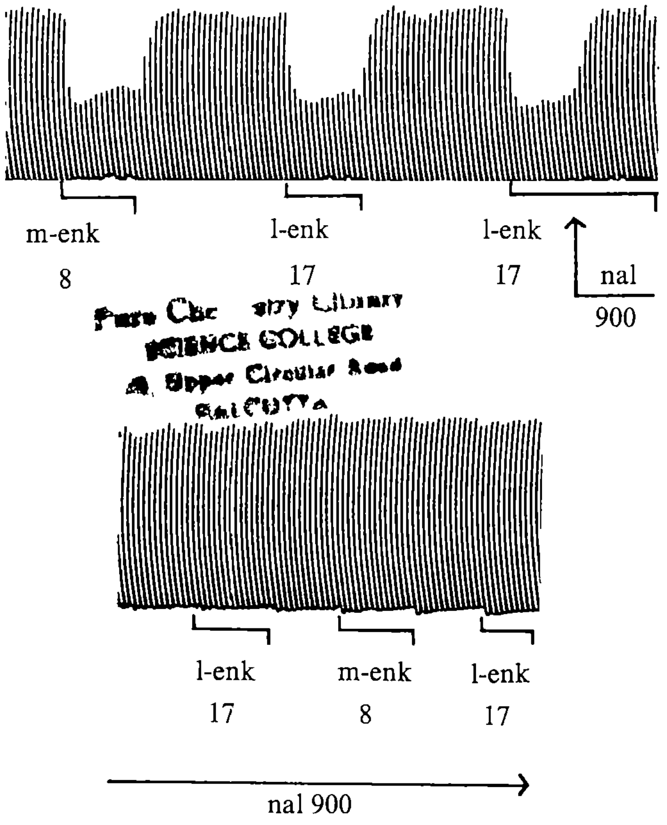

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Fig. 3 The depressant effects of methionine-enkephalin (m-enk) and leucine-enkephalin (l-enk) on the electrically induced contractions of the mouse vas deferens, and the antagonist effect of naloxone (nal) which was present throughout the lower tracing. The numbers give concentrations in nM. The concentration of naloxone (900 nM) was chosen to give complete reversal of the inhibition; 275 nM caused an 80% reversal.

The discovery in the brain of two endogenous pentapeptides with potent opiate agonist activity raises a number of pertinent questions which cannot be adequately dealt with in this paper. It will now be possible, however, to test the hypothesis[19] that these peptides act as neurotransmitters or neuromodulators at synaptic junctions.

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Mechanism of catalysis of hydrocarbon reactions by platinum surfaces

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Atomic height steps and kinks in steps have been identified on platinum surfaces as distinct catalytic sites, where C–C, C–H and H–H bond scissions occur during the reactions of hydrocarbons. The active catalyst surface is covered with a carbonaceous overlayer whose properties affect both the reaction rates and the production distribution. A model for the catalysis of hydrocarbons on platinum surfaces is proposed which incorporates the effects of the low coordination number active sites and the atomic surface structure.

WE have studied the correlation between the activity of platinum crystal surfaces carrying out a variety of catalytic reactions of hydrocarbons, and their atomic surface structure and surface composition. We report here the identification of distinct atomic sites on the platinum surface where H–H, C–H and C–C bond breaking predominantly occur. The hydrogen-deuterium exchange reaction[1] provided information about the H–H bond breaking, whereas the C–H and C–C bond breaking activity was determined by monitoring simultaneously the rates of dehydrogenation and hydrogenolytic ring opening of cyclohexane to produce benzene and *n*-hexane, respectively[2]. Experimental evidence also indicates that the catalytically active surface is covered with a carbonaceous overlayer which forms as a result of partial dehydrogenation of hydrocarbons[2,3]. The overlayer is either ordered or disordered, dependent on reactant and clean platinum surface structure, the ordering influencing both the product distribution and the rate of surface reactions.

The surface structures of the clean platinum single crystals and of adsorbed molecules were studied by low-energy electron diffraction, and the surface composition by Auger electron spectroscopy[4]. The rates and product distributions in the various chemical reactions were measured by a quadrupole mass spectrometer at low pressures[3] and a gas chromatograph at high pressures[5]. The surface area of the single crystal catalyst samples was about 1.0 cm^2. Reaction rates on the catalyst surfaces as low as 10^{-15} m cm^{-2} s^{-1} were readily detectable.

Active sites on platinum surfaces

Three types of platinum surface, which are distinguishable by their reactivities, are shown schematically in Fig. 1. The low Miller Index Pt($\bar{1}11$) surface (Fig. 1*a*) has a very low density of surface imperfections, $<10^{12}$ cm^{-2}, as compared with the number of surface atoms, 1.5×10^{15} atoms cm^{-2}. The high Miller Index Pt($\bar{5}57$) surface (Fig. 1*b*) is characterised by a stable surface structure with 18% of the surface atoms in monatomic height steps[6]. The Pt($\bar{6}79$) surface (Fig. 1*c*) must have, in addition to high step density, a high density of kinks in the step or atoms of lower coordination since the ($\bar{6}79$) plane does not lie between two close packed planes as does the ($\bar{5}57$) plane [($\bar{1}11$), and ($\bar{0}01$)][4]. Thermal stability of high Miller Index surfaces has been systematically investigated, and their relative stability as a function of crystal orientation, and relative activities in various hydrocarbon reactions have been determined[7]. The different surface sites are distinguishable by their coordination number, that is, their number of nearest neighbours, ranging from kink atoms with only 6, to atoms in (111) orientation terraces with 9.

Molecular beam scattering indicates the reaction probability (number of reactive collisions per total collisions with surface) for H–D exchange increases by four orders of magnitude from $<10^{-5}$ on the Pt($\bar{1}11$) surface to ~0.1 on a highly stepped platinum surface, Pt($\bar{3}55$)[1]. The presence of a high concentration of atomic steps was necessary to detect the formation of HD on single scattering at low pressures, and the amount of HD formed was proportional to the step density.

We have also found that the dehydrogenation of cyclohexane and cyclohexene to benzene occurs at an appreciable rate only on stepped platinum surfaces[2,8]. Figure 2 demonstrates this by showing the dependence of the rate on step density for the latter reaction. The rate of cyclohexane dehydrogenation to benzene is constant as long as there are steps (tested as low as 7% steps) on the catalyst surface, but is almost an order of magnitude lower on the Pt($\bar{1}11$) surface, as seen in Fig. 3. Thus atomic steps seem to be the preferred surface sites for breaking H–H and C–H bonds.

The relative rates of dehydrogenation and hydrogenolysis for cyclohexane can best be monitored by the ratio of benzene to *n*-hexane in the reaction products. As shown in Fig. 3, the rate of benzene production is independent of step and kink density, while *n*-hexane production increases slowly with step density and rapidly with kink density. The rate of *n*-hexane production per kink site is determined by the slope of the line in Fig. 3*b*, which gives 1×10^{-20} mol s^{-1} per kink atom. This is almost an order of magnitude higher than the slope in Fig. 3*a* which corresponds to 1.2×10^{-19} mol s^{-1} per step atom. The observed low hydrogenolysis activity on the type of stepped surfaces represented in Fig. 1*b* may be caused by thermally generated kinks in the steps[9]. Since the formation of *n*-hexane and other hydrogenolysis products must be the result of C–C bond scissions, it seems that kinks are very effective in breaking C–C bonds as well as C–H and H–H bonds. Thus we have been able to identify two active sites of lower coordination number on platinum surfaces, steps with C–H and H–H and kinks in steps with C–C, C–H and H–H bond breaking activities.

The carbonaceous overlayer

During the experiments, the catalyst surfaces were always covered with a carbonaceous overlayer[2–4]. The coverage was almost independent of pressure ranging from 10^{-4} to 10^5 Pa (unpublished results of this laboratory) but varied markedly with temperature and the molecular weight of the saturated hydrocarbon reactant molecules[2]. The higher the temperature and the reactant molecular weight, the higher the coverage, reaching monolayer amounts for cyclohexane at 725 K or *n*-heptane at 575 K. Unsaturated hydrocarbons, such as ethylene or benzene, form complete monolayers at all temperatures and pressures, and double layers in certain conditions[7,10], with the reactant adsorbed on the overlayer. The overlayer consists of partially dehydrogenated hydrocarbon species[2–4] formed from the dissociation of the reactants. This carbonaceous overlayer may be ordered or disordered depending on the platinum surface structure, the nature of the reactant

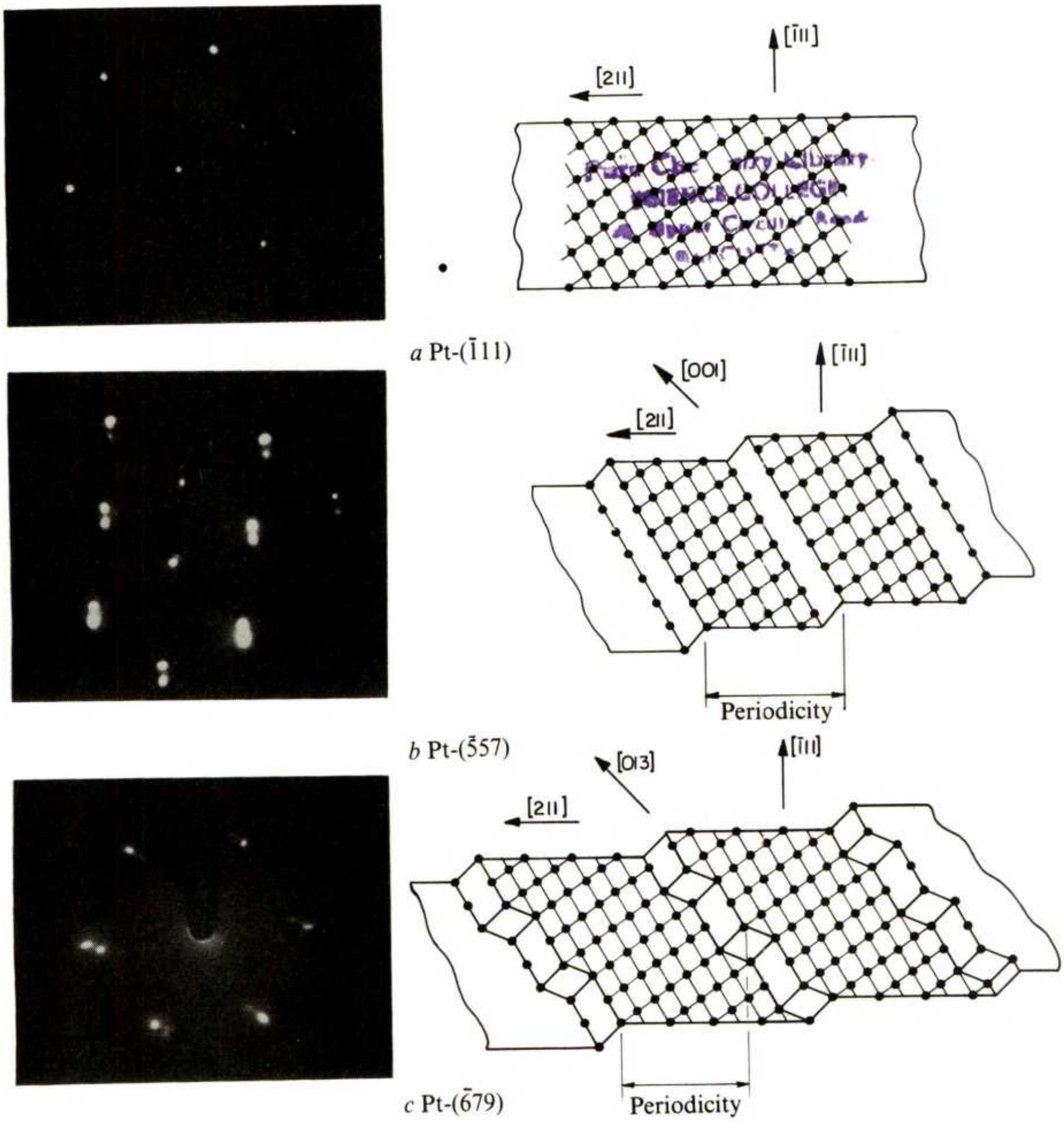

Fig. 1 Low energy electron diffraction patterns and schematic representations of the mean surface configurations of three representative platinum single crystal catalyst surfaces. *a*, Pt(1̄11) surface containing $< 10^{12}$ defects cm^2; *b*, Pt(5̄57) surface containing 2.5×10^{14} step atoms cm^{-2}, with an average spacing between steps of 6 atoms; *c*, Pt(6̄79) surface containing 2.3×10^{14} step atoms cm^{-2} and 7×10^{13} kink atoms cm^{-2} with an average spacing between steps of 7 atoms and between kinks of 3 atoms.

and the hydrogen-hydrocarbon ratio used in the experiment. Several reactions are very sensitive to the presence of ordering on the overlayer[2]. Cyclohexene conversion to benzene is poisoned unless the overlayer is ordered, and *n*-heptane to toluene conversion occurs only in the presence of an ordered overlayer[3]. Other reactions like the hydrogenolysis of cyclohexane occur readily even in the presence of a disordered overlayer.

Model of the active platinum catalyst

As a result of these experimental observations we would like to propose an atomic model of the platinum surface active in the catalysis of hydrocarbon reactions (see Fig. 4). The surface is heterogeneous both in atomic structure and in composition. The steps and kinks are the active sites where C–H and H–H and C–C, C–H and H–H bond breaking occur, respectively[4].

Although steps and kink sites exhibit higher binding energy for both hydrogen and hydrocarbons, the greatly increased rate of dissociation of hydrogen at steps[1] may keep these sites active. Even though these sites are areas of high catalytic activity, the product desorption probably occurs from areas covered with the carbonaceous overlayer. Diffusion on, and desorption from, the areas covered by the carbonaceous overlayer is likely since the binding energy of products should be lower there. The structure of the carbonaceous overlayer is important in more complex reactions like dehydrocyclisation of *n*-heptane to toluene and in reactions where rapid deactivation of the catalyst occurs. Thus, two types of structure sensitivity are to be distinguished, step structure sensitivity and overlayer structure sensitivity.

Since much of the structure sensitivity of the catalytic reactions reported in the literature has been deduced from the dependence (or independence) of the reaction rate on the mean particle size of dispersed catalyst particles[11,12], caution should be exercised in interpreting the mean particle-size dependence of the reaction rate in terms of our model. Some of the structural features may vary in proportion to changes of particle

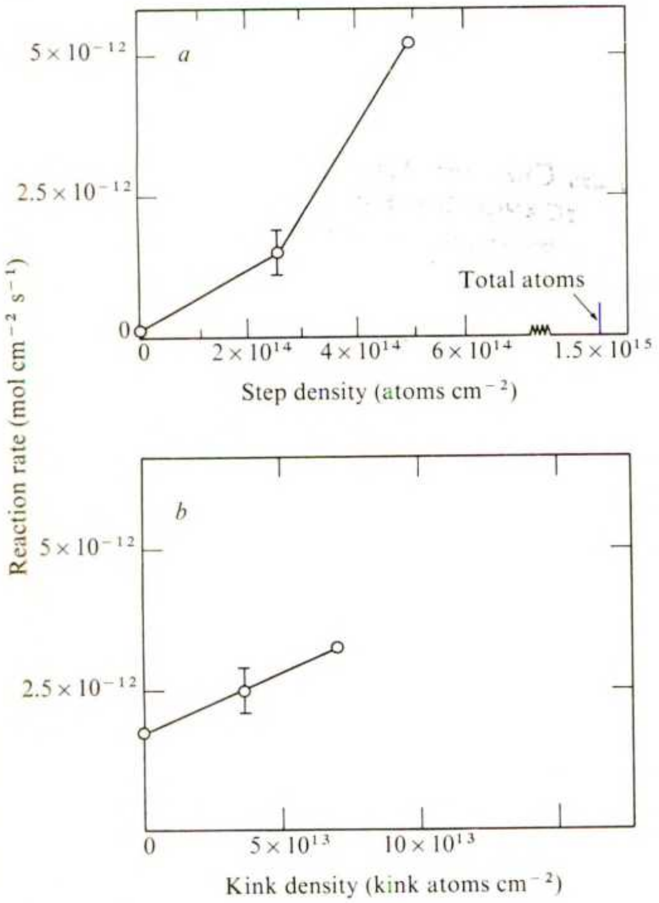

Fig. 2 Initial rate of cyclohexene dehydrogenation to benzene on platinum single crystal catalysts with *a*, increasing step density and *b*, increasing kink density at a constant step density of 2.5×10^{14} step atoms cm^{-2}. The reaction conditions are 5×10^{-6} Pa of cyclohexene, 1.0×10^{-4} Pa of hydrogen and 423 K catalyst temperature. Hydrogen–hydrocarbon ratio 20 : 1

size (perhaps step density) while other structural features (step–kink site ratio, overlayer structure) may not. Our experimental observations support the active site concept originated by Taylor[13] for the importance of the heterogeneity of atomic surface structure in catalysis. Our model also accommodates the structure sensitivity–insensitivity reaction classification proposed by Boudart[12].

It should be noted that in our studies of platinum catalytic activity, the effect of the porous material used to support the highly dispersed metal particles in actual catalysts has not been taken into account. The support may influence the concentration and structure of the atomic steps and the carbonaceous overlayer. Most of the catalytic reactions that are attributed to the platinum in supported platinum catalysts[14] have, however, been reproduced on the single crystal surfaces in the absence of the support. Thus, we do not expect that our model of the platinum catalyst is markedly changed when any effects of the support are taken into account.

Although many of our reaction studies on single crystal surfaces are carried out at both low[2,3] (10^{-4} Pa) and at high[5] pressures (10^5 Pa) the rate determining steps in the mechanisms of surface reactions might be expected to change with the more than eight orders of magnitude change in pressure. Whether the structural features that control the platinum catalysis of hydrocarbon reaction will be less influential at high pressure than at low pressure will have to be determined by future experiments.

The causes for the unique bond breaking activity of low coordination number sites (steps, kinks) on surfaces has been the topic of theoretical concern[15–18]. The extent to which the variation of chemical behaviour at surface irregularities is the property of transition metals other than platinum is yet to be determined. Although palladium stepped surfaces exhibit higher heats of hydrogen chemisorption than the low Miller Index surfaces[19], gold, a non-transition metal, exhibits oxygen and hydrocarbon chemisorption properties that are identical on both stepped and low Miller Index surfaces[20].

The model of the catalytically active platinum surface also explains the effect of selective poisoning that influences the product distribution in hydrocarbon reactions so markedly. An impurity, a second component or a poison like sulphur, present in small concentrations, will block first the kink sites that are of the highest binding energy on the heterogeneous surface, drastically reducing the C–C bond breaking activity (hydrogenolysis rate), and only moderately affecting the catalytic reactions where only C–H and H–H bond breaking is required (dehydrogenation or hydrogenation). This way selectivity in product distribution may be achieved.

It should be pointed out that the two important structural features that characterise the heterogeneous platinum catalyst surface—atomic steps and carbonaceous overlayers—also emphasise the relationship of heterogeneous catalysis to homo-

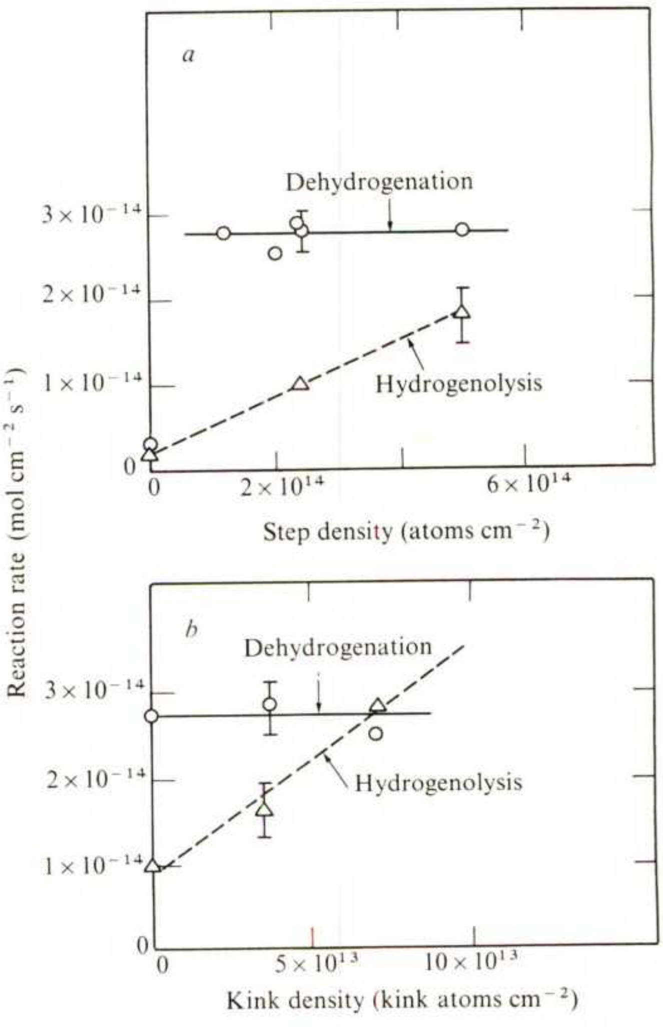

Fig. 3 Initial steady state rates of cyclohexane dehydrogenation to benzene (○) and hydrogenolysis to *n*-hexane (△) on platinum single crystal catalysts with *a*, increasing step density and *b*, increasing kink density at constant step density of 2.4×10^{14} step atoms cm^{-2}. The reaction conditions are 5×10^{-6} Pa of cyclohexane, 1.0×10^{-4} Pa of hydrogen and 423 K catalyst temperature. The rate of dehydrogenation is constant at 2.8×10^{14} mol cm^{-2} s^{-1} with steps present on the surface but is less (0.3×10^{14} mol cm^{-2} s^{-1}) on the Pt($\bar{1}11$) surface (0.0 step density). The rates of hydrogenolysis per surface site at the slope of the lines representing hydrogenolysis and are 1×10^{-20} mol s^{-1} per kink atom and 1.2×10^{-19} mol s^{-1} per step atom. Hydrogen–hydrocarbon ratio 20 : 1.

geneous and enzyme catalysis. The catalytic activity or bond breaking ability of clusters of atoms along steps or near kinks on the heterogeneous metal surface may be correlated with the reactivity of homogeneous systems with a single metal atom or metal clusters surrounded by ligands. The metal–ligand catalysts will break and form H–H and C–H bonds as do the metallic clusters, but generally lack the ability to break C–C bonds which is possessed by the highly uncoordinated kink atoms on metallic clusters. The presence of ordered carbonaceous overlayers which apparently are necessary to perform the complex rearrangement of large molecules, for example the dehydrocyclisation of *n*-heptane to toluene, or to prevent catalyst deactivation may be correlated with the structure sensitivity and specificity so important in complex enzyme reactions.

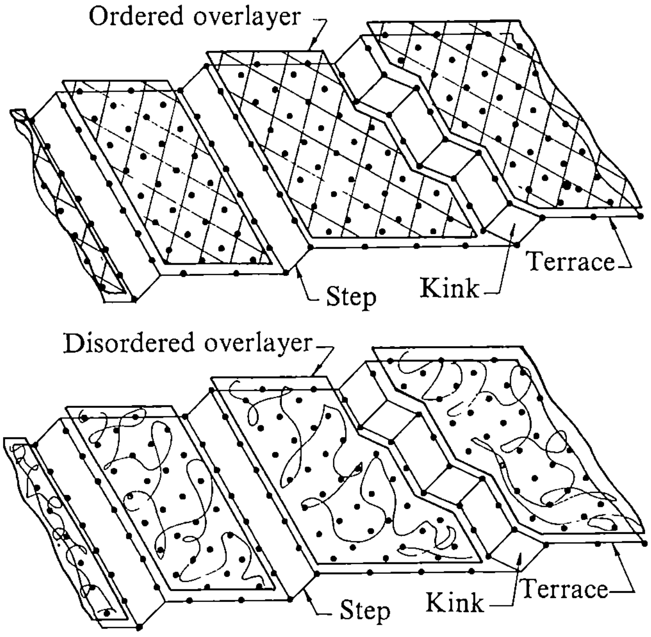

Fig. 4 Model of active platinum catalyst surface with a full carbonaceous overlayer showing exposed catalytic sites.

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letters to nature

A spherical accretion model of Cyg X-1

THE binary X-ray source Cyg X-1 is widely believed to be a black hole, and most current models for explaining its X-ray spectrum[1] assume an accretion disk. The reason for requiring a disk, rather than a spherical accretion flow, is that earlier investigations of the properties of spherically accreting black holes seemed to indicate that, for the mass transfer rates required to give the observed luminosities of 10^{37}–10^{38} erg s^{-1}, the cooling time of the gas would be so short compared to the free-fall time that the gas would arrive essentially cold at the Schwarzschild radius, and there being no solid surface to transform the infall kinetic energy into thermal energy, very little of this would be radiated away[2–4].

In fact, spherical accretion on to a black hole can convert gravitational energy into radiation with an efficiency comparable with that of disk systems[5]. Earlier results, indicating that if

$$L = \eta \dot{M}[GM/r_S] \tag{1}$$

(where $r_S = 2GM/c^2$ is the Schwarzschild radius) then $\eta \ll 1$, neglected to take account of the spherical inflow being necessarily subject to magnetohydrodynamic and plasma turbulence, and that this provides a viscous dissipation mechanism which heats the gas far more efficiently than the $p\mathrm{d}V$ work. That the spherical accretion flow must be turbulent follows, if nothing else, from the fact that the large scale shear $\sigma \sim (GM/r^3)^{1/2}$ leads to a growth of whatever initial frozen-in magnetic field is present[2], up to values $H^2/8\pi \gtrsim GM\rho/r$ (Since $\dot{H}^2 \propto r^{-4}$ but $GM\rho/r \propto r^{-5/2}$). This near-equipartition value cannot be exceeded, because the magnetic energy can only be generated at the expense of the gravitational energy; reconnection of field lines must occur, leading to plasma instabilities and to turbulence being continually regenerated. Although equipartition magnetic fields were considered in previous spherical calculations, the heating arising from the turbulent viscous dissipation, and the Joule heating arising from the field line reconnection were not included. That such dissipative heating must occur, however, is necessary for the same reason as in disk models, namely, that inflow could not proceed without it: the excess magnetic energy must be dissipated before the magnetic pressure tries to halt the collapse, and the angular momentum associated with random transverse turbulent motions has to be carried away (for example by collision between blobs) to allow the gas to move inwards.

We may estimate the magnitude of the heat input rate per unit volume, Γ, from the condition that the turbulent energy content of a volume bounded by r and $r/2$ must be dissipated in the turbulent time scale (~ free-fall time), or that the magnetic energy content in the same volume be dissipated in the Alfvén time scale. If both of these energy forms are near equipartition with the gravitational energy (which makes the Alfvén time approximately equal to the free-fall time as well) then this condition is

$$\Gamma r^3 t_{ff} \sim \bar{\rho} v_t^2 r^3 \sim \left(\frac{H^2}{8\pi}\right) r^3 \sim \frac{\beta}{4}\left(\frac{GM\bar{\rho}}{r/2}\right) r^3 \tag{2}$$

where ν_t is turbulent velocity, t_{ff} is free-fall time and $\beta \lesssim 1$. The heat input rate is therefore

$$\Gamma \sim 4\times10^{32} r^{-4} n_a \beta \left(\frac{M}{10M_\odot}\right)^3 \left(\frac{\nu}{2\times10^8 \mathrm{cm\ s^{-1}}}\right)^{-3} \mathrm{erg\ cm^{-3}\ s^{-1}} \quad (3)$$

where M is the mass of the central compact object, n_a is the baryon number density at the accretion radius $r_a = GM/\nu^2$, $\nu = (\nu_w{}^2+\nu_s{}^2)^{1/2}$ with ν_w = wind velocity and ν_s = sound velocity at r_a, and the free-fall law for the density was assumed, $\rho \propto r^{-3/2}$. The inclusion of this dissipative heating has the effect of making the efficiency factor η of equation (1) close to unity for densities such that the cooling times are shorter than the free-fall times, quite unlike the result of previous calculations. The fact that the turbulent field is continuously excited by field line reconnection and plasma instabilities, and that this magnetic-turbulent energy must be dissipated, ensures that gravitational energy is converted into heat at all radii down to the Schwarzschild radius without the need to invoke a solid surface to halt the flow. This mechanism should therefore have a role in neutron star, or white dwarf accretion as well. Most of the luminosity comes from the regions nearest to the Schwarzschild radius r_S, since

$$L(r) \sim 4r^3\Gamma(r) \propto n_a M^3 r^{-1} \quad (4)$$

and provided that $t_{cool} \lesssim t_{ff}$ at least near to r_S an efficiency η close to unity is ensured by the fact that $H^2/8\pi$, or $\rho\nu_t{}^2$ must build up to equipartition as a consequence of flux-freezing. Thus, spherical accretion flows can be just as efficient as disk systems, for accretion on to a black hole.

The main observational properties of Cyg X-1 can be reproduced by such a spherically accreting black hole, without going into a very detailed calculation. We take as an example $M = 10\ M_\odot$, and adopt the accretion radius $r_a = 3.3\times10^{10}$ cm, with a wind velocity $\nu_w \sim 10^3$ km s^{-1} and a sound velocity ν_s appropriate to a temperature $T \sim 3\times10^7$ K. To have a luminosity $L \sim 10^{37}$ erg s^{-1}, from equation (1) we require a density at the accretion radius $n_a \geqslant 10^{10}\ \eta^{-1}$ cm^{-3}, or $M \simeq 4\pi r_a{}^2 \nu\rho_a \gtrsim 5\times10^{16}\ \eta^{-1}$ g s^{-1}. In the absence of any Compton scattering, the X-ray spectrum is due to bremsstrahlung, and the spectrum is $L_\nu \propto r^3\varepsilon_\nu(r) \propto \nu^{-1/2}$, where $\varepsilon_\nu(r) \propto n(r)^2 T(r)^{-1/2}$ is the differential emissivity per unit volume. Since Cyg X-1 has a spectrum that, at least part of the time, is steeper than this, it seems necessary to invoke Compton scattering to reproduce the spectrum. In the disk calculation of Shapiro *et al.*[6], it is shown that, for an electron temperature $T_e \sim 10^9$ K, and for the Compton scattering parameter $y = (4kT_e/m_e c^2)\tau_{es}{}^2 \simeq 1$ (where τ_{es} is the Thomson optical depth) then a solution of the photon transport equation in phase space (Kompaneets equation) is able to reproduce the spectrum $L_\nu \sim \nu^{-1}$ as required by observations, provided that a copious supply of soft photons [energy 50 eV $\lesssim h\nu_\lambda \lesssim$ 5 keV] is present. Since, however, the cyclotron mechanism, which is the likeliest source of soft photons, has a ground harmonic frequency far below 50 eV, they have to invoke either recapture of radiation from regions further out, or postulate arbitrary soft photon sources in the gas. We can adapt this Compton scattering calculation to the spherical case, by choosing similar parameters, without in fact having to invoke an arbitrary soft photon source, or recapture. The cyclotron mechanism produces photons in the higher harmonics as well, and in fact most photons are not at the ground harmonic, but rather at the harmonic ν_m at which the cyclotron opacity $\tau_{\nu,cy}$ drops below unity, and this is usually a very high harmonic. Below ν_m, the cyclotron mechanism gives a Rayleigh–Jeans spectrum $L_\nu \propto \nu^2$, and above ν_m it drops abruptly. Choosing $n_a = 2\times10^{12}$ cm^{-3}, we have $n = 4\times10^{17}$ cm^{-3} and $\tau_{es} \simeq 2.5$ at a radius $r = 2\ r_S = 10^7$ cm. The equipartition average magnetic field is $\langle H\rangle = 3.5\times10^7$ and the cyclotron frequency $\langle\nu_H\rangle = 10^{14}$ Hz at that radius, and using the expression[7] for the quasi-continuous cyclotron opacity coefficient $\kappa_\nu = (4\pi^2\nu_P{}^2/c\nu_H)\ A \exp(-a\nu/\nu_H)$, one finds that $\nu_m \simeq 4\times10^{15}$ Hz at an assumed temperature $T \simeq 10^9$ K. These values are based on the mean values $\langle H\rangle$ and $\langle\nu_H\rangle$, but in fact the field line reconnection and dissipation should lead to inhomogeneities, so that the blobs doing most of the emission and absorption of photons should have values of H and ν_H higher than the average. It is very difficult to estimate the mean square fluctuations, but it seems quite likely that $\nu_m \sim 1\text{–}2\times10^{16}$ Hz, $h\nu_m \gtrsim$ 40–80 eV as required for input photons in the Compton scattering process. The spectrum above ν_m would then be a power law in the energy, with index ~ -1 up to an energy $h\nu \sim kT \sim 10^2$ keV and dropping off above that. The total luminosity from Compton cyclotron radiation is $L \sim$ few $\times 10^{37}$ erg s^{-1}, indicating that the assumed temperature of $T \simeq 10^9$ K is approximately the right temperature for radiating away the thermal energy produced by the dissipation–reconnection mechanism of equation (3) with $10^{-1} \lesssim \beta \lesssim 1$. Since most of the radiation arises from within a few Schwarzschild radii, the millisecond time variability of Cyg X-1 is explained by the usual light-travel time arguments, the total luminosity corresponds to what is observed, and the overall features of the spectrum up to hard X-rays, are as satisfactory as in the latest improved disk models. The intensity and spectrum changes (for example the 1971 downward transition, with a harder spectrum) may be explained by changes in the accretion rate. A lowering by a factor ~ 4 of the mass transfer rate would proportionally reduce the total luminosity and the Thomson optical depth. The less efficient Compton cooling leads to a higher temperature near the Schwarzschild radius, and to a harder spectrum with a flatter power law exponent.

A spherical model may be necessary in Cyg X-1 if the mass transfer in the binary system is not fed by Roche lobe overflow, but rather by a wind[8,10,11]. Even if there was enough angular momentum to form a disk, or even a marginal, unstable disk[9], the thin disk approximation used would not be valid in the hard X-ray emitting regions, since height $\sim$ radius. In such circumstances, spherical geometry seems a closer representation of reality than a disk, and furthermore does not require spin flipping of a specialised nature, extraneous photon sources or uncoupled electron and proton temperatures (although these last two could be accommodated). A spherical model therefore seems to afford a certain economy of assumptions.

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B0924+30 an unusual radio galaxy

THE radio source B0924+30 (ref. 1) has been studied as part of a systematic survey of radio galaxies of relatively low ($<10^{41}$ erg s^{-1}) radio luminosity. The radio source is identified with a 14 mag elliptical galaxy with a redshift $z = 0.0266$ (refs 2 and 3). A 12-h observation of this radio source was made with the Westerbork Synthesis Radio Telescope[4] at 1.4 GHz in September 1974, and Fig. 1 is a contour plot of the brightness distribution obtained. The resolution is 23″ in right ascension and 46″ in

Table 1 Positions and flux densities for the components of B0924+30

	External component	*a*	*b*	*c*
Position RA	—	09h 25m 50.0±0.1s	09h 24m 24.8±0.1s	09h 23m 33.9±0.1s
dec.	—	30° 20′ 45±2″	30° 07′ 18±2″	29° 58′ 33±3″
Angular distance from galaxy	±5.5′	14′	8′	22′
Flux density at 1.4 GHz (10^{-29} W m^{-2} Hz^{-1} mJy)	221	51±3	54±3	62±3
Flux density at 0.6 GHz (mJy)	530	84±13	76±12	71±14
Spectral index	−1.0	−0.6±0.2	−0.4±0.2	−0.2±0.2
Component diameter	4′	< 10″	< 10″	< 10″
Linear distance from galaxy (kpc)	±126	322	187	508
Luminosity (W Hz^{-1} sr^{-1})	$10^{22.2}$	$10^{21.5}$	$10^{21.5}$	$10^{21.6}$
Position angle of vector between nucleus and component	55±15°	54.5°	53°	52°

declination. The r.m.s. noise level in the map is 0.5 mJy. For a single 12-h observation the map is distorted by elliptical grating rings of semiaxis 10′×20′. The grating rings from many of the small diameter sources have been removed, but the distortion from the extended part of the radio galaxy remains and is the cause of the low level negative regions to the north and south of the galaxy. No correction for the primary beam attenuation, which reaches a factor of 2 at the edge of the map, has been applied. The position of the nucleus of the elliptical galaxy is RA = 09 h 24 min 55.1 s, dec. = 30°12′15″, and is indicated by the + in Fig. 1.

The extended source is in itself interesting—it shows clearly an S-shaped structure with respect to the galaxy, already noticed in less striking form in some other radio galaxies, notably in Cyg A[5] and 3C98 (ref. 7).

Even more intriguing are the three point-like sources (*a*, *b*, and *c*) in Fig. 1, which have almost equal intensity and lie on a line passing through the nucleus of the elliptical galaxy at the same position angle as the extended components. In addition to the 1.4 GHz map we have a set of 7 short (7 min) observations made with the Westerbork Synthesis Radio Telescope at 0.6 GHz. Although the map made from these observations has much higher noise (r.m.s. ~ 4 mJy) than the 1.4 GHz map, it is adequate to estimate the flux densities of the components. The three point-like sources have spectra which are somewhat flatter than average. Table 1 gives the positions and flux densities of all components, and the linear dimensions and luminosity assuming a Hubble constant of 100 km s^{-1} Mpc^{-1}.

Precise alignment between the compact components and the nucleus of the parent galaxy has already been seen in other cases (for example, Cyg A[5] and DA240 (ref. 6)), but two features are unusual in this case:

First, the compact components lie well outside the region of extended emission. Although rarer, this may also have been observed in some other radio galaxies, such as 3C61.1 (ref. 7). Here, however, the radio emission extends all the way to the compact components. Second, there are three, rather than two, compact objects involved, and this has not been seen previously.

Of course the most important question to ask is whether this unusually striking alignment necessarily implies a physical association. A definite answer to this question cannot depend on the application of statistics after the event, but must await either the discovery of further similar configurations, or direct evidence for a connection such as an emission bridge.

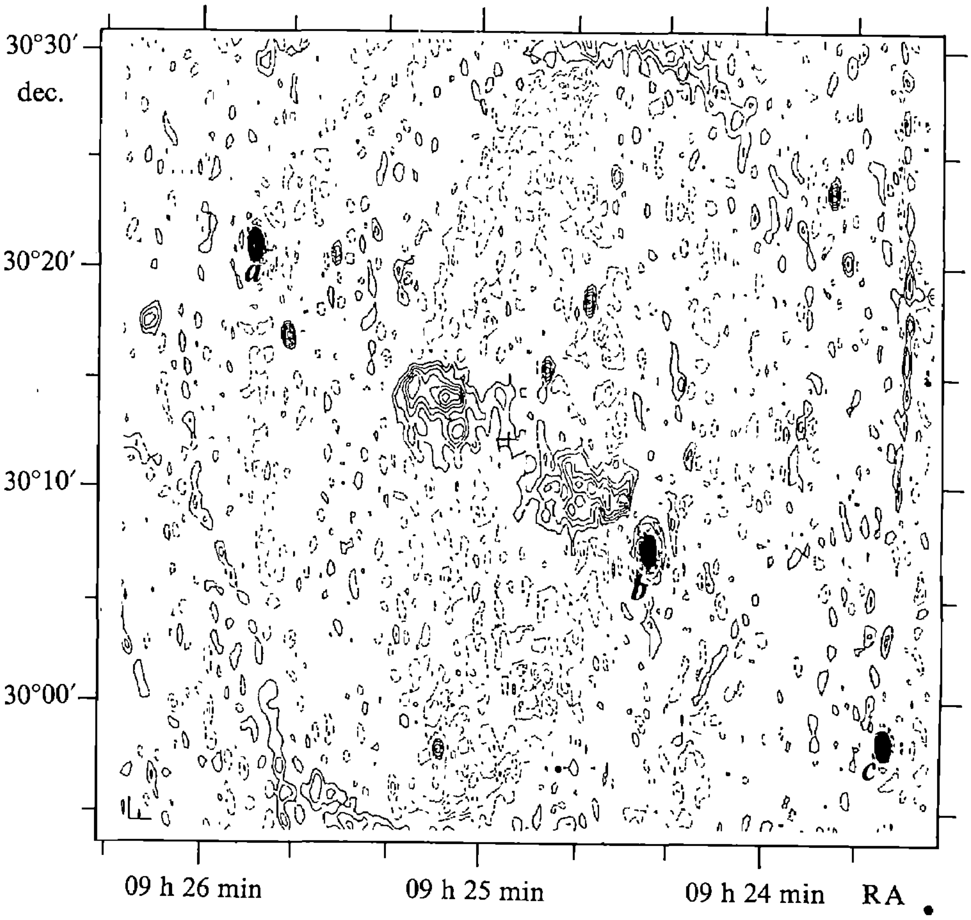

Fig. 1 Contour map of the vicinity of the radio source B0924+30 at 1.4 GHz. The contours are at equal intervals in steps of 1.0 mJy per synthesised beam area (0.6 K). Negative contours are broken and the zero contour is not shown. The + indicates the position of the 14 mag galaxy.

Table 2 Probability of chance alignment

Probability	Event
1.3	One source $\geqslant$ 50 mJy in primary beam area.
0.022	A second source lying within $\pm 1.5°$ of the line from the first source to the galaxy.
0.022	A third source within $\pm 1.5°$ of the same line.
0.17	Probability that the line through the three sources agrees with the position angle of the extended source to within $\pm 15°$.
0.16	Probability that all three sources have equal intensity within a factor of 1.4 (observed ratio is 1.2).

Combining these we obtain a final probability of 1×10^{-5}.

No similar configurations have been previously seen, but there has been a strong observational selection against such objects. There is selection against low luminosity objects in complete radio source surveys—the components in our case are rather weak compared with the noise level in most maps, and they are sufficiently far away from the parent galaxy that either the identification would not be noticed, or the area mapped would be too small to contain them. We know of ~ 10 observations of low luminosity radio galaxies in which such a configuration would have been seen, but was not.

We have searched for any possible bridge connection between these components by smoothing the 1.4 GHz map to lower resolution. A possible connection can be seen between component *b* and the tip of the extended source but this is not significantly ($\sim 1.5\sigma$) above the noise. Further observations are planned at 0.6 GHz to improve the sensitivity to weak extended structure and at 5.0 GHz to improve the resolution on the compact components.

Since we have no other case of this type, and no strong evidence for connections, it is necessary to comment on the estimated probability of occurrence of such an alignment. The density of radio sources at this flux level at 1.4 GHz is 1.3×10^4 sr^{-1} (estimated from the observations of Katgert *et al.*[8]); hence, it is not unlikely that some sources at this level will be seen in the 10^{-4} sr area of our primary beam. We give in Table 2 the steps in a calculation of the probability of an alignment by chance so that the reader can judge for himself the merit of each step.

To make this estimate as meaningful as possible we have used only probability calculations with some *a priori* justification, namely: First, the alignments seen between compact components and the nucleus of the galaxy in Cyg A, DA240 are $\leqslant 1.5°$. Second, other compact components related to the parent galaxy lie within the angle subtended by the extended component. Third, the median intensity ratio for normal double radio sources is 1.4 (refs 9 and 10).

The positions of the three aligned components have been searched on the prints of the Palomar Sky Survey, and in one case, source *b*, there is a stellar image with ultraviolet excess near the plate limit. The position of this object is: RA = 09 h 24 min 24.8 ± 0.1 s, dec. = 30° 07′ $18.0 \pm 0.8''$, which is within 2″ of the radio position. This is within the errors in the radio position and would normally be considered as a rather certain QSO identification.

Clearly if this association of the three compact radio components with the extended radio source and its parent galaxy is a physical association it will have important implications for the theory of formation of double radio sources. There are three rather than the more usual two components; the compact components lie well outside of the region of extended emission; they are aligned with the inner part of the S-shaped extended source rather than the outer part. On the other hand, without further examples of the same type it would be premature to exclude the possibility that one or more of these components are unrelated field sources.

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Selection of quasars by variability

The first step in gleaning cosmological information from quasars is to determine their spatial distribution and luminosity function, based on well defined, complete samples[1,2]. This idealised situation applies to a great extent in the case of radio quasars. For the majority of quasars, which are radio quiet, however, existing samples based on ultraviolet excess are not only incomplete, but are dominated by colour selection effects[3]. The relative importance of these effects depends on redshift, and, in particular the detection of high redshift quasars is extremely difficult[4]; consequently other methods of selection of radio-quiet quasars and BL Lac-type objecrs have been suggested[5,6]. Here, one such method based on optical variability is considered, with particular regard to a sample of 13 objects observed by van den Bergh *et al.* (BHP)[6].

Variability has long been known to be an important characteristic of quasars[7]. Its use as a major selection criterion is, however, jeopardised not only by contamination from other variable objects, but also by uncertainties about the nature of the subgroup of quasars which are selected.

There are three main types of variable objects which will contaminate a 'variability selected' sample. These are luminous variable stars (mainly RR Lyrae), low luminosity late M-type flare stars and N-type galaxies[8]. Provided samples are obtained in directions well out of the galactic plane, the number of RR Lyrae and other luminous variable stars should be very low. The number of late M-type flare stars and N galaxies are, however, virtually independent of direction, and also increase with fainter limiting magnitude. The contaminating effects of these objects can, to a certain extent, be mitigated by the introduction of further weak colour selection. In particular late M-type flare stars would have extreme red colours, and thus could be removed from a sample, although some quasars might be missed in this way. A closer study of the light curves of all the objects in a sample would result in the RR Lyrae and other luminous variable stars being recognised as such, although this could select against quasars whose variation resembled these objects.

A more fundamental problem is that of the nature of the quasars selected and their relationship to quasars in general. The probability of a quasar being in a sample depends on the form of its luminosity curve with time. For example, periodic variation would be detected more easily than flare behaviour. Also, small variations would not be detected, especially in objects whose brightness was close to the limiting magnitude of a sample. The final, and possibly most serious effect, arises if variability is correlated with any other quasar parameters (for example, redshift), in which case redshift-dependent selection effects of an unknown nature would be operating. Note that any correlation found[9] between variability and high

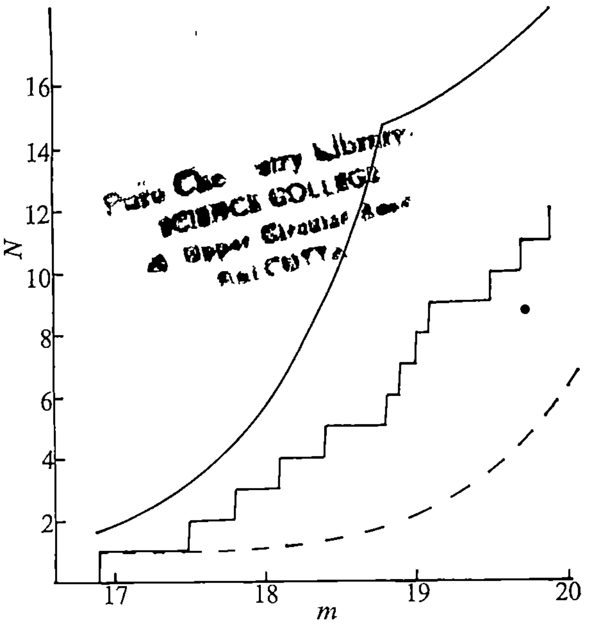

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Fig. 1 The total number of objects in the BHP sample as a function of apparent magnitude (step function). The lower curve is the expected number of contaminating objects, and the upper curve includes the number of radio-quiet quasars, based on ultraviolet excess selection methods.

intrinsic luminosity is probably a selection effect. Since the number of quasars increases rapidly with decreasing apparent brightness, a variable quasar in a sample is much more likely to be a much fainter quasar which has brightened its apparent magnitude (and absolute magnitude) therefore being an overestimate. It would thus seem that variable quasars were, on average, of higher luminosity that non-variable quasars, giving rise to the correlation. This effect can be seen in Weistrop's sample[10], where quasar candidates which were initially selected by colour, and then studied for variability, tended to decrease in magnitude after the initial selection.

In our variability selected sample, the numbers of contaminants were estimated as follows:

Luminous variable stars. The calculations were based on Field I of the Palomar–Groningen variable star survey (PG1)[11]. The survey is centred on galactic coordinates $l=0°$, $b=29°$, and has a limiting magnitude of ~ 19 mag, although the sample is less complete towards fainter magnitudes. Variable stars are detected by blinking plate pairs, and allowance for incompleteness can be made using the statistics of the number of stars detected on 1,2,. . .10 plate pairs. The BHP field is centred on $l=120°$, $b=-20°$ and a modified selection criterion is employed, in which objects are only deemed variable if they appear on at least two independent plate pairs out of the total of 21. This enables objects to be selected to a much fainter limiting magnitude, while at the same time avoiding contamination of the sample by spurious variables caused by grain statistics. The number of variables in PG1 is estimated to be 130, of which 42 are detected on at least two out of ten plate pairs. This number should not increase at fainter magnitudes, since all the variables within the Galaxy should be bright enough to be detectable. An extrapolation of these numbers for the 21 plate pairs of the BHP field, and a reduction by a factor ~ 10 to take into account the relative directions of PG1 and the BHP field results in 0.9 luminous variables, or approximately half of the variables being detected in the 6.2 square degrees of the BHP field[12]. Although the reduction by a factor of 10 is very uncertain, the number of luminous variables expected should not be more than 2.0.

Late M-type flare stars. Since most stars of type M5 and later exhibit flares of more than 0.5 mag variation[13], and five out of the 100 nearest stars are of this type[14], the number of these stars per square degree down to a limiting magnitude m_V is

$$4.1\times10^{-7}\times10^{0.2(3m_V-27.6)} \qquad (1)$$

where an absolute magnitude of M_V ~ 14 mag has been assumed[14]. About half of the variables would be detected, so that down to $m_V=19.2$ mag (the approximate limiting magnitude of the BHP sample) ~ 1.2 late M-type flare stars would be detected. This figure is a lower limit since the number of flare stars with $M_V > 16$ is unknown. Also, since these stars would be within about 100 pc of the solar neighbourhood, they will be distributed fairly randomly over the sky.

N-type galaxies. According to W. Sargent (private communication), there are about 1.0 N galaxies per square degree down to the limit of the Palomar Sky Survey prints. If it is assumed that ~ 25% are detected, then 1.5 N-type galaxies are expected in the BHP field.

Quasars. We use the number–magnitude relation based on quasars found by the ultraviolet excess method of selection[15]

$$\log N = -0.27+0.6\,(m_V-17.8);\quad m_V<18.8^m \qquad (2)$$

where N is the number of quasars per square degree and a mean value of $m_B-m_V=0.2$ mag has been employed. N is assumed constant for magnitudes fainter than $m_B=19$ mag ($m_V=18.8$ mag)

Figure 1 shows the total number of objects in the BHP sample as a function of apparent magnitude (step function). The lower curve is the number of contaminating objects and the upper curve includes the number of quasars according to equation (2). The BHP sample therefore contains approximately 65% quasars (1.2 per square degree) and provides about half the number of quasars generated by ultraviolet excess selection methods, but without the accompanying redshift-dependent selection effects. Variability selection therefore can provide comparable numbers of radio quiet quasars and BL Lac-type objects as other methods of selection, provided that the limiting magnitude is within the range 19 to 20 mag and, as in the BHP sample, directed away from the galactic plane. The number of quasars detected can be increased still further either by using a greater number of plate pairs, or by extending the limiting magnitude so that variable objects of up to 20 mag can be detected on one plate pair.

It should be noted that two of the objects in the BHP sample (denoted j and k) are separated by only 22″. They may be variable stars or, if quasars, similar to the interesting quasar pair 1548+115 (ref. 16).

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Photosynthesis of organic compounds in the atmosphere of Jupiter

THE substances responsible for the red-brown colours of the atmosphere of Jupiter are the source of much debate Initially it was suggested that the coloured materials are HCN oligomers and polymeric organic nitriles which are formed during electrical storms on Jupiter[1,2]. This model has been criticised on two grounds: first it seems unlikely that there could be sufficient lightning on Jupiter to account for the intense red-brown coloration and second, the electrical storms could only take place in the lower atmosphere of Jupiter, where condensation of water vapour takes place, and therefore there would have to be some means of transporting the compounds above the NH_3 ice clouds[3]. Another postulate is that the colour comes from elemental sulphur and polysulphides formed by the photolysis of H_2S (ref. 4). Organic compounds may also be produced when H_2S is photolysed in the presence of CH_4 and the other hydrocarbons present in the Jovian atmosphere[5,6]. Since neither H_2S nor NH_4SH has been detected in the upper atmosphere of Jupiter it is necessary to postulate either that the rate of H_2S photolysis is much faster than its rate of transport to the upper atmosphere, or that sunlight reaches the lower atmosphere through gaps in the ammonia ice clouds and the sulphur and polysulphides so formed are then transported to the upper atmosphere. A third postulate suggests that both organic compounds and polysulphides may be formed by a bombardment of the atmosphere with high energy protons and electrons[7].

Table 1 Reactants and products from the photolysis of CH_4, NH_3, H_2, and He mixtures*

Reactants	Pressure (mm Hg)	mol	Reactant consumed or photoproduct (mol)
NH_3	30	2.87×10^{-3}	1.8×10^{-3}†
CH_4	95	9.15×10^{-3}	1.5×10^{-3}†
H_2	474	45.33×10^{-3}	$\sim 4 \times 10^{-3}$‡§
He	60	5.75×10^{-3}	
N_2			0.83×10^{-3}‡
HCN			7×10^{-7}‡
N_2H_4			1×10^{-8}‡
C_2, C_3 hydrocarbons			‖

* The results given are an average of three runs in which the gaseous mixture was photolysed in a 1.8-l flask for 1 h using the low pressure mercury lamp, and the extent of NH_3 decomposition was measured spectrophotometrically using a 10-cm cell. CH_4, N_2 and the C_2 and C_3 hydrocarbons were detected and analysed by mass spectrometry[9]. He was used as the standard in the quantitative mass spectral analysis. HCN was detected and determined colorimetrically[10,11] after the reaction products were dissolved in H_2O. Both methods indicated the same yield. N_2H_4 was analysed by specific colour test [12]. Care was taken to use N_2H_4-free NH_3. All reactants were shown to be pure by mass spectral analysis.

† Amount consumed

‡ Amount formed.

§ An approximate yield. Wide variation in yield was observed

‖ No quantitative analyses were performed.

We reported that the NH_3 in the upper atmosphere of Jupiter is subject to rapid photodecomposition[8]. Here we observe that an efficient conversion of CH_4 to hydrocarbons and HCN takes place when NH_3 is photolysed in the presence of CH_4, H_2, and He using a 184.9-nm light source (see Table 1). Of particular significance is the observation that photolysis of 1 molar equivalent of NH_3 results in the loss of 0.84 molar equivalent of CH_4. The CH_4 undoubtedly reacts with hot hydrogen atoms formed by the photolysis of NH_3 to NH_2 and H. The 92% yield of N_2 demonstrates that 8% of the NH_3 is not converted to N_2. Presumably this NH_3 is converted to organic amines and nitrile derivatives such as HCN. The high yield of H_2, approximately 30% greater than the stoichiometric amount expected on NH_3 photolysis, is consistent with hot hydrogen atom reactions[13–15]. This increased yield of H_2 reflects the H_2 formed by the condensation of CH_4 to higher molecular weight hydrocarbons.

These results demonstrate that NH_3 photolysis results in the very efficient conversion of CH_4 to more complex organic compounds in the upper atmosphere of Jupiter. This pathway for hydrocarbon synthesis explains how ethane and acetylene are formed in the upper atmosphere of Jupiter[16] since C_2 hydrocarbons were detected in our experiments. From the results of our photochemical studies we predict the presence of HCN, NH_2NH_2 and higher hydrocarbons in the Jovian atmosphere above the NH_3 clouds. Further condensation of these photoproducts may yield organic compounds responsible for the red-brown coloration in the upper Jovian atmosphere. Since this postulate requires neither the synthesis of the chromophoric compounds in the lower Jovian atmosphere, nor their transfer to the upper atmosphere, it is not subject to the criticisms discussed already for models requiring the use of electrical discharges or H_2S photolysis.

Although these results are primarily of significance to photochemical reactions of the Jovian planets and some of their satellites, they may also be applicable to the primitive Earth. Our previous results suggest that any atmospheric NH_3 would have been rapidly photodestroyed in the 1–10 Myr period after a significant atmosphere developed[17,18]. NH_3 photolysis could have resulted in the efficient formation of organic molecules if this primitive atmosphere contained significant amounts of CH_4 or CO along with the NH_3 (ref. 19).

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Variation in time of mercury anomalies at the Mid-Atlantic Ridge

MERCURY concentrations vary markedly from place to place in the world ocean[1,2], we report here variations with time in bottom waters over the Mid-Atlantic Ridge at the FAMOUS area. Three sets of water samples from the axial rift valley have been examined since our original report of anomalous Hg in that region[3]. In 17 months Hg concentrations varied from 1,100 to 70 ng l^{-1} (Table 1; Fig. 1).

Samples have been analysed using the cold vapour atomic absorption method (without modification)[2]. Water was handled and stored according to the techniques recommended by Bothner and Robertson[4]. Sampling containers, procedures and personnel were provided by us on the first, third and fourth occasions. On the second, we obtained samples of water from polyethylene containers of known history allowing us to accept the data as a lower limit for mercury in the water at that time. Every sample was taken within 1.5 miles of 36°49′25″N, 33°15′40″W; bottom depths averaged 2,530 m. Samples from up to 230 m above

Table 1 Sample data

Date	Hg (ng l^{-1})*	Number of samples	Distance above bottom (m)	Sampling
October 1973	1,080 (1,420–870)	14	10	M. M. Jones from N.R.L. towed fish, Niskin bottle rosette, USNS MIZAR (T-AGOR–11)
July 1974	250 (362–195)	6	1—ALVIN 0–180 KNORR profile	Dr H. Holland, Harvard University, WHOI, RN KNORR, ALVIN
October 1974	67 (83–58)	7	0–230 profile	This work; Niskin bottle hydrocast, USNS MIZAR (T-AGOR–11)
March 1975	78 (161–12)	5	0–175	R. Nekritz, D. Greenwalt, Niskin bottle hydrocast USNS BARTLETT (T-AGOR–13); Dr P. Taylor, SSOB, J. Ackerman, NavOceano

*Median value (range in parentheses).

the bottom were used since vertical profiles showed no identifiable trends.

Our results show clearly that large Hg anomalies can exist at certain times whereas normal oceanic concentrations prevail at others. We have not determined the source or sink for these anomalies. One attractive hypothesis is that there are intermittent hydrothermal injections from active ridge spreading centres[3,5]. Local temperature anomalies of up to 0.1 °C have been reported for the FAMOUS site[6], similar in magnitude and character to those presented by Scott *et al.*[7], for another Mid-Atlantic Ridge site at 26°N, 45°W. Whatever the source, no information is yet available concerning the rate of injection and dissipation. Therefore, we cannot distinguish between a single injection with slow removal rate, or multiple injections with more rapid removal. Mercury has been found in close combination with the particulate phases in natural waters[8], so removal is probably attributable to advection and/or the sedimentation of particles over periods of days to months, though mercury enrichment of nearby sediments has not been established as it has for sediments of the East Pacific Rise[9].

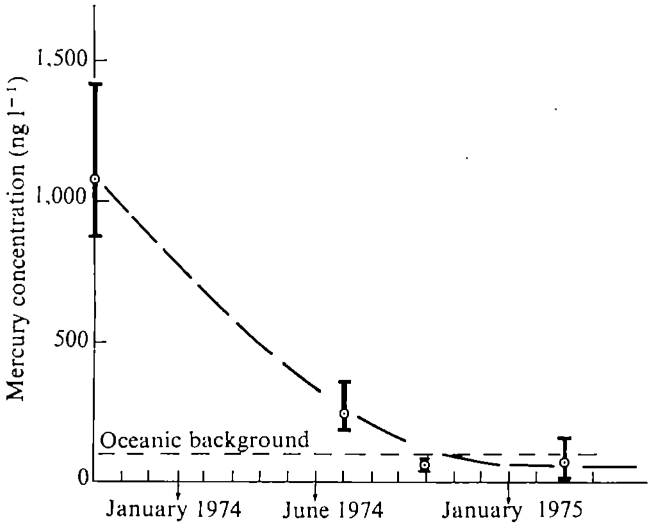

Fig. 1 Variation in time of the mercury content of bottom water around the Mid-Atlantic Ridge: – – – – –, median value; bars indicate range.

The October 1973 samples, which showed drastically increased mercury concentrations, did not exhibit increased Fe, Mn or F concentrations[3]. These elements are often enriched in areas of tectonic activity. The 'normal' amount of these elements in seawater is from 10 to 1,000 times higher than that of Hg; so considerably larger inputs would be needed to show similar perturbations. Furthermore, new inputs of Fe and Mn to seawater are removed to the sediments very rapidly and very close to the injection point[10].

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Origin and ancient history of gneisses older than 2,800 Myr in Lewisian Complex

THIS work records new field observations (to be discussed in detail elsewhere) which suggest that the pre-2,800 Myr history of the Lewisian Complex in the type localities of Scourie and Laxford, north-western Sutherland, was closely comparable with that of Godthåb, western Greenland. In western Greenland Precambrian rocks have been grouped into older (Amitsoq) and younger (Nuk) gneisses[1], separated in time by the emplacement of metamorphosed mafic–ultramafic Ameralik dykes. Supracrustal rocks and layered cumulitic intrusions, probably younger than the Amitsoq gneisses but older than the Nuk gneisses are also present and have all been varyingly affected by high-grade amphibolite or granulite facies metamorphism. Although the Lewisian Complex contains gneisses which, together with supracrustal and layered cumulitic igneous rocks, have suffered the widespread granulite facies metamorphism dated at about 2,800 Myr BP and can be compared with those of western Greenland[1,8], equivalents of the Amitsoq and Nuk gneisses have previously not been distinguished.

All the Lewisian outcrops discussed here are cut by dolerite dykes or their metamorphosed equivalents, belonging to the early Proterozoic Scourie Dyke Swarm. Between Badcall and Scourie, where the dykes retain igneous minerals, the gneisses are at granulite facies and yield dates of about 2,800 Myr (ref. 3).

In that region the oldest identified rocks are gneisses, in which layered basic igneous intrusions have been emplaced. The gneisses also contain medium-grained, homogeneous,

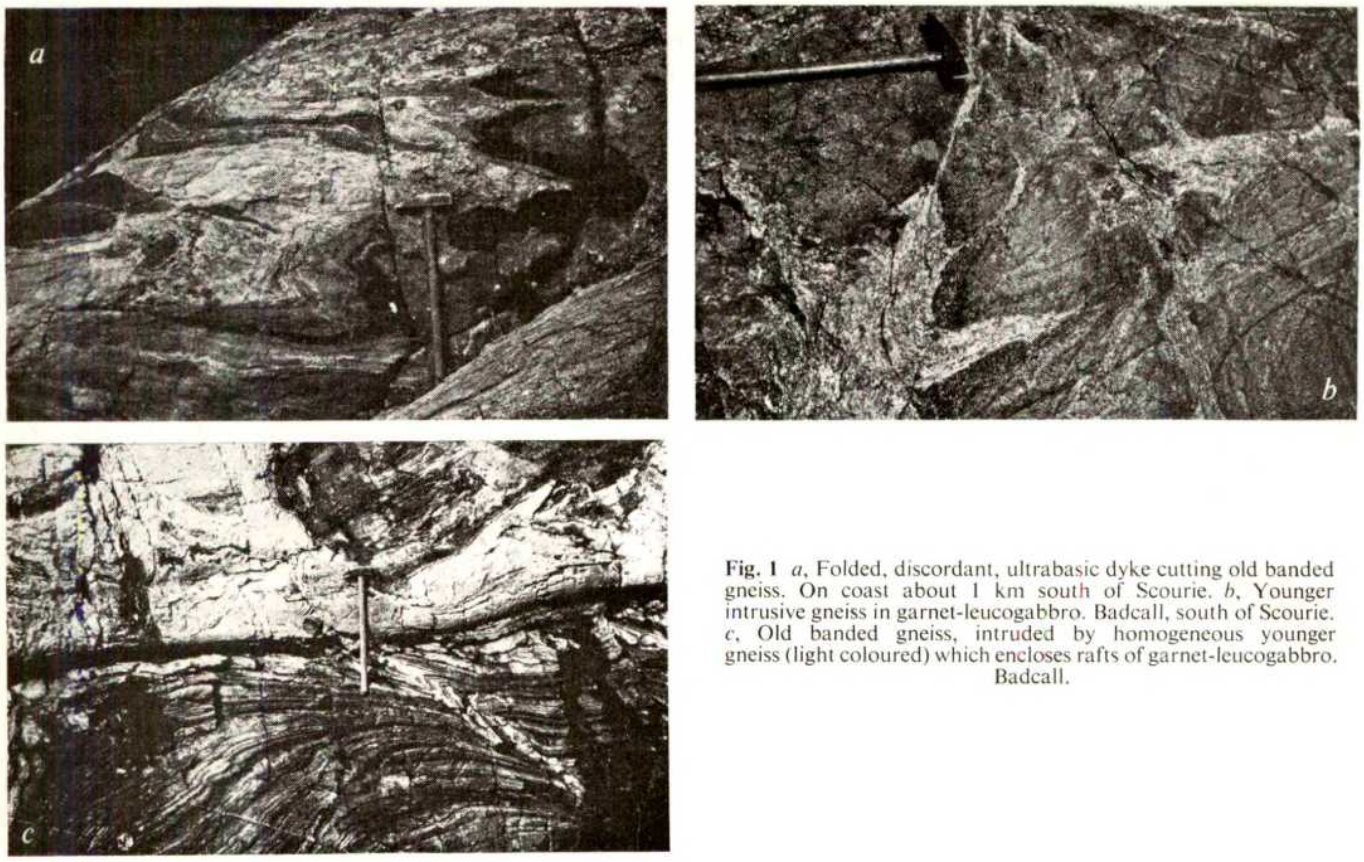

Fig. 1 *a*, Folded, discordant, ultrabasic dyke cutting old banded gneiss. On coast about 1 km south of Scourie. *b*, Younger intrusive gneiss in garnet-leucogabbro. Badcall, south of Scourie. *c*, Old banded gneiss, intruded by homogeneous younger gneiss (light coloured) which encloses rafts of garnet-leucogabbro. Badcall.

ultramafic–mafic layers, which are often highly deformed, broken up and migmatised. Where later deformation and migmatisation have had least effect, the layers retain discordant relationships to irregular banded gneiss and have a dyke-like habit. Where these metadykes are least deformed it is clear that the bulk of the oldest recognised rocks are leucocratic medium to coarse grained, quartzofeldspathic gneisses with which are intercalated dark pyroxene-bearing bands up to 1 m thick. Large feldspars up to 2 cm in diameter occur in the darker gneiss lithologies. Ultramafic layers and pods up to a few metres thick consisting mainly of pegmatitic green pyroxenes also occur in these old gneisses, although metadykes have not been observed to cut the ultrabasic pegmatites. Although the mineralogy of the leucocratic gneiss indicates a broad similarity to gneisses of igneous origin, no conclusive evidence bearing on the origin of these rocks has so far been recognised and they could represent acid–intermediate supracrustals metamorphosed in plutonic conditions.

Deformed varieties of the old gneisses in association with occasional graphite-bearing and sulphide-bearing garnet–sillimanite gneisses, which contain thin concordant ultramafic layers that may represent metadykes, form the rocks in which the layered intrusions were emplaced[2].

Rocks affected by the 2,800 Myr granulite facies metamorphism in which the metadykes and their deformed equivalents have not been recognised include the widely distributed layered cumulitic igneous sheets and abundant quartzofeldspathic gneisses which commonly retain intrusive relationships with the layered basic sheets and all the older rocks.

Three distinct intrusive phases of gneisses younger than the layered intrusions have been recognised. The earliest is a dark, intermediate phase which, although relatively uncommon and without convincing intrusive relationships, contains fragments of the older rocks, and has nowhere been found to contain early metadykes. The younger phase, intrusive into the first phase, is a light granitic rock which locally retains a coarse, probably igneous texture. Pink perthite–quartz veins and discordant sheets up to about 1 m thick constitute the last intrusive phase. They occur throughout the complex but seem commonest at the margins of, and within, the layered basic rocks; sheets within the layered basic intrusions are usually thinner than the same bodies in the nearby gneisses. Although many of the younger phases indicate injection of the new material into the older rocks, at sites of locally high strain pegmatitic patches of the younger phase are commonly in close association with the old gneisses which contain the early metadykes.

All of these rocks show the effects of the overprinted, Scourian granulite facies metamorphism and must, therefore, be older than, or contemporaneous with, that event. North of Loch Laxford the Lewisian Complex has also suffered Laxfordian amphibolite facies metamorphism[4] which affects the Scourie dykes. The effects of Scourian granulite facies metamorphism are, however, still occasionally retained. The younger phases of quartzofeldspathic gneisses north of Loch Laxford contain, and are intrusive into, fragments of layered basic rocks and supracrustal rocks, and in the field they are in every way comparable with the main younger intrusive phase in the granulite terrain south of the Laxford Front. The youngest phase is also represented north of the Laxford Front, as pink microcline–quartz sheets in the remnants of the layered basic rocks.

The oldest gneisses make up less than 50% of the complex between Badcall to Scourie, and occur as thin strips, up to about 100 m wide in the younger phases. The strips are often directly in contact with layered basic igneous sheets and are conformable with the structure marked by them. The younger, intrusive, quartzofeldspathic phases occur as veins and discordant sheets in all the older rocks and as large units which contain isolated, disrupted inclusions of the older rocks. The impression is that the strips of old rocks are seen in interrupted stages of incorporation into the younger phases. The oldest gneisses (containing metadykes), have not been recognised north of the Laxford Front and their scarcity or absence in that region could explain some of the geochemical differences known between the Scourian and Laxfordian complexes[5]. Major bodies of successive younger intrusive phases are often located along interfaces between the older rock types, especially at the interface between units of old gneisses and of gneisses of supracrustal origin. Although the integrity of the ancient interface remains, marked by the layered basic sheets, the older gneiss has been almost completely incorporated into the younger gneisses. The long-lived influence of that ancient interface[6] probably represents the junction between an extremely ancient basement (pre-Scourian) of old gneisses containing metadykes and the supracrustal rocks.

The field evidence suggests that gneisses younger than the layered basic intrusions have been derived both from injected material and from the local remobilisation and incorporation of gneisses older than the layered intrusions. The sequence of pre-2,800 Myr events (Table 1) is directly comparable with that determined for the western Greenland and Labrador portions of the North Atlantic craton[7,8]. The gneisses older than the layered intrusions are comparable with the Amitsoq gneisses and those younger than the layered intrusions with the Nuk gneisses of the Godthäb region, western Greenland.

Table 1 Outline sequence of Archaean events in the Scourie region

8 Intrusion of Scourie Dykes about 2,200–2,400 Myr BP.
7 Deformation and amphibolite facies metamorphism commencing about 2,600 Myr BP.
6 End of granulite facies metamorphism, about 2,800 Myr BP.
5 Younger gneiss group:
  iii Pink perthite–quartz veins common near contacts of layered bodies. Probably syntectonic to earliest recognised structures developed on Laxford lineament.
  ii Light coloured homogeneous quatrzofeldspathic gneisses. Main intrusive phase, igneous textures probably locally preserved.
  i Dark coloured intermediate phase. Relationships uncertain but metadykes not found.
4 Layered cumulitic igneous sheets of the type associated with stratiform anorthosites of Outer Hebrides.
3 Metamorphosed dykes. Ultramafic varieties up to tens of centimetres wide.
2 Supracrustal rocks ranging from garnet–sillimanite gneisses and quartz–magnetite rocks to basic amphibolites.
1 Older gneiss group; quartzfeldspathic gneisses, intermediate pyroxene gneisses and ultramafic pods.

The recognition in the granulite facies terrain south of the Laxford Front of gneisses whose field relationships and relative ages resemble those of the Amitsoq group is in apparent conflict with strontium and lead isotope studies[9] which suggest that the 2,800-Myr old terrain of the Lewisian Complex does not incorporate a significant proportion of reworked older crust (100–200 Myr old) equivalent to the 3,700-Myr old gneisses of western Greenland. These studies have led to the idea that a significant addition of crustal material from an upper mantle source, in the form of volcanic or plutonic rocks, occurred about 3,000–2,800 Myr BP in the Lewisian Complex. The recognition of old gneisses separable in the field from several generations of intrusive gneisses in the rocks of the granulite facies, and the possible derivation of younger gneisses by the local regeneration of old gneisses, do not support this postulation.

The absence of dates significantly older than 2,800-Myr and the isotopic ratios recorded from Lewisian rocks, could be partly explained by the ubiquitous nature of the younger intrusive phases, the youngest phase of which may be almost contemporaneous with the granulite facies metamorphism. Since those rocks form more than half the complex and provide most of the relatively homogeneous material suitable for sampling, the results obtained may be based mainly on an analysis of them alone.

To assist in future correlation between these units and others throughout the craton, the older gneisses and the supracrustal rocks are here respectively termed (after localities) the 'Uamhaig Gneisses' and the 'Claisfearn Supracrustals'. The metadykes are termed the 'Shios Dykes' and the suite of younger intrusive gneiss phases are termed the 'Clach Boga Gneisses'.

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Arctic palaeosalinities during late Cainozoic time

DURING the late Cainozoic, alternations between interglacial and glacial conditions, manifested by waning and waxing of continental ice sheets coincided with the rise and fall of seawater temperatures. Our palaeontological and oxygen isotope data indicate that although the major oceans and low latititude seas underwent large temperature variations, ~ 5–10 °C, Arctic water temperatures remained near freezing point for at least the past 3 Myr, the time interval represented by the longest studied cores. We have determined palaeotemperatures from the ratio of left to right coiling *Globigerina pachyderma*[1–3] and calculated palaeosalinities using the oxygen isotope palaeotemperature expression of Epstein *et al.*[4].

Our data suggest that normal and low salinity cycles were superimposed on temperature fluctuations. The lowest temperatures prevailed during the cold pulses of the Brunhes normal polarity and late Matuyama reversed polarity epochs and, somewhat higher temperatures and lower salinities a little more than about 1 Myr ago, in Matuyama time are indicated by planktonic foraminifera. Ice-rafted detritus throughout the Alpha Rise cores (Fig. 1) suggests that high latitude glaciation was under way 3 Myr ago. Ages have been estimated by interpolations and extrapolations based on radiometric dates and on close interval biostratigraphic and lithologic correlations between cores, some of which already have an established palaeomagnetic stratigraphy[3,5].

On the basis of faunal and sedimentray characteristics three major climatic units can be distinguished: Unit III include sediments deposited earlier than 2.4 Myr BP, during the Gauss reversed polarity epoch; the fauna constitutes less than 1% of the coarse fraction (> 63 μm, Fig. 2). Planktonic foraminifera are dominated by sinistrally coiled *G. pachyderma* (94–100%), and related forms, some of which are corroded[3,5]. Arenaceous foraminifera dominate the benthonic foraminiferal assemblages. Sedimentation rates which were lower than those of the present day, and/or corrosive deep water may account for the selective solution of the less resistant calcareous tests. The impoverished character of the fauna precludes a palaeoclimatic reconstruction of this period.

The boundary between units II and III is marked by simultaneous lithological and faunal changes and occurs near a magnetic reversal (ref. 5; Fig. 2). The lithological change from dark brown, Mn and Fe oxide-rich, silty-lutite below, to tan silts with higher percentages of coarse, ice-rafted detritus above the boundary, is accompanied by faunal alterations. Sediments of unit II deposited about 2.4–0.7 Myr ago, during the Matuyama reversed polarity epoch, are poor in both foraminifera and in Fe and Mn oxides, but contain one layer rich in

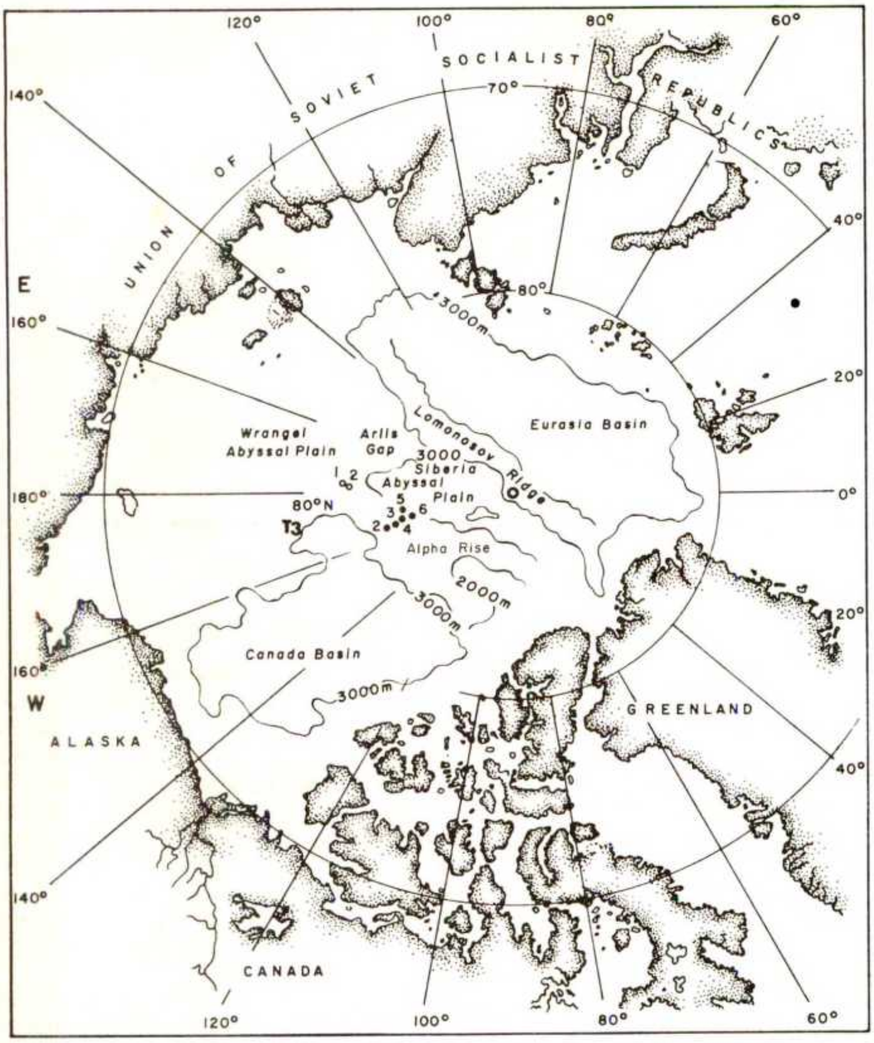

Fig. 1 Bathymetric map of the Arctic Ocean based on the *Geological Map of the Arctic* (1960). T3, Location of cores T3/67-3, 4, 9, 11, 12; PM, cores with know palaeomagnetic stratigraphy; RI, Radioisotopically dated core. Dark and open circles, locations of previously studies cores (refs 3 and 5).

Table 1 Data from collected fauna

Cores	Depths (cm)	Species analysed	δ¹⁸O	δ¹³C	Temperature** (°C)	Salinity ‰	δ¹⁸O water	Melt water (%) ($\delta^{18}O = -30$)
T3/67-3*	98–101	*Cibicides wüellerstorfi* *Eponides tener umbonatus*	+3.93	+0.89	−0.5	33.8	−0.8	2.5
T3/67-3	98–101	*G. occlusa*	+3.16	+0.64	−1.7	32.5	−1.9	6.3
T3/67-3	98–101	*G. pachyderma*	+3.24	+0.78	−1.7	32.6	−1.8	6
T3/67-3	160	*G. pachyderma*	+1.00	−2.92	−1.0	30.4	−3.7	12.3
T3/67-3	160	*G. occlusa*	+1.30	+0.29	−1.0	30.8	−3.4	11.3
T3/67-3	160	*G. pachyderma* (*5 chambered*)	+0.96	+0.30	−1.5	30.0	−4.0	13.4
T3/67-3	203–205	*G. quinqueloba*	+0.17	−0.86	−1.5	29.1	−4.82	16.1
T3/67-3	219–222	*G. pachyderma*	+0.89	+0.30	−1.5	29.9	−4.1	13.7
T3/67-3	219–222	*G. occlusa*	−1.05	−1.46	−1.0	26.2	−7.3	24.4
T3/67-3	219–222	*G. pachyderma* (*5 chambered*)	+0.73	+0.31	−1.5	29.8	−4.2	14.2
T3/67-3	219–222	*C. wüellerstorfi*	+2.60	−0.04	−0.8	32.2	−2.2	7.3
T3/67-3	245–248	*G. pachyderma*	+1.12	+0.15	+1.5	31.5	−2.8	9.3
T3/67-3	245–248	*G. occlusa*	+0.73	+0.16	+1.5	30.9	−3.3	11.0
T3/67-3	250	*G. occlusa*	+1.15	+0.01	+1.5	31.5	−2.8	9.3
T3/67-3	250	*G. pachyderma*	+1.26	+1.5	+1.5	31.6	−2.7	9.0
T3/67-4†	80	*G. quinqueloba*	+1.42	−0.42	−1.0	30.6	−3.5	11.9
T3/67-11‡	Top	*G. pachyderma*	−1.21	−0.19	−1.5	27.5	−6.2	20.7
T3/67-11	6	*G. pachyderma*	+1.65	+0.70	−1.8	30.6	−3.5	11.7
T3/67-11	10	*G. pachyderma*	+0.62	+0.45	−1.0	30.2	−3.9	13.0
T3/67-11	10	*G. pachyderma*	+1.26	+0.01	−1.7	30.3	−3.8	12.7
T3/67-12§	0–2	*G. pachyderma*	+0.16	−0.04	−1.0	29.4	−4.6	15.6
T3/67-12	192	*G. quinqueloba*	−0.03	−0.95	−1.0	29.0	−4.8	16.3
Dst A2¶	0	*G. pachyderma*	+1.42	+0.56	−1.0	30.6	−3.5	11.9
Dst A2	7	*G. pachyderma*	+2.29	+0.67	−1.7	31.5	−2.7	9.3
Dst A2	39–40	*G. pachyderma*	+1.95	+0.82	−1.7	31.0	−3.1	10.5
Dst A2	75	*G. pachyderma*	+2.21	+1.10	−1.7	31.6	−2.8	9.0
Dst A2	180	*G. quinqueloba*	+1.15	−1.26	+1.5	31.4	−2.8	9.5
Dst A2‖	180	Composite sample	+1.42	−0.42				

* T3/67-3; 79°11′N; 175°09′W; 2,285 m water depth.

† T3/67-4; 79°22.7′N; 174°46′W; 1,760 m water depth.

¶ Dst A2; 83°52′N; 168°12′W; 1,521 m water depth.

‖ 180 cm composite sample: Planktonic, benthonic foraminifers, ostracods, pelecypods.

** Temperature estimated mainly from the coiling direction of *G. pachyderma;* see text for details.

‡ T3/67-11; 79°34.9′N; 172°30′W; 2,810 m water depth.

§ T3/67-12; 80°21.9′N; 173°33′W; 2,867 m water depth.

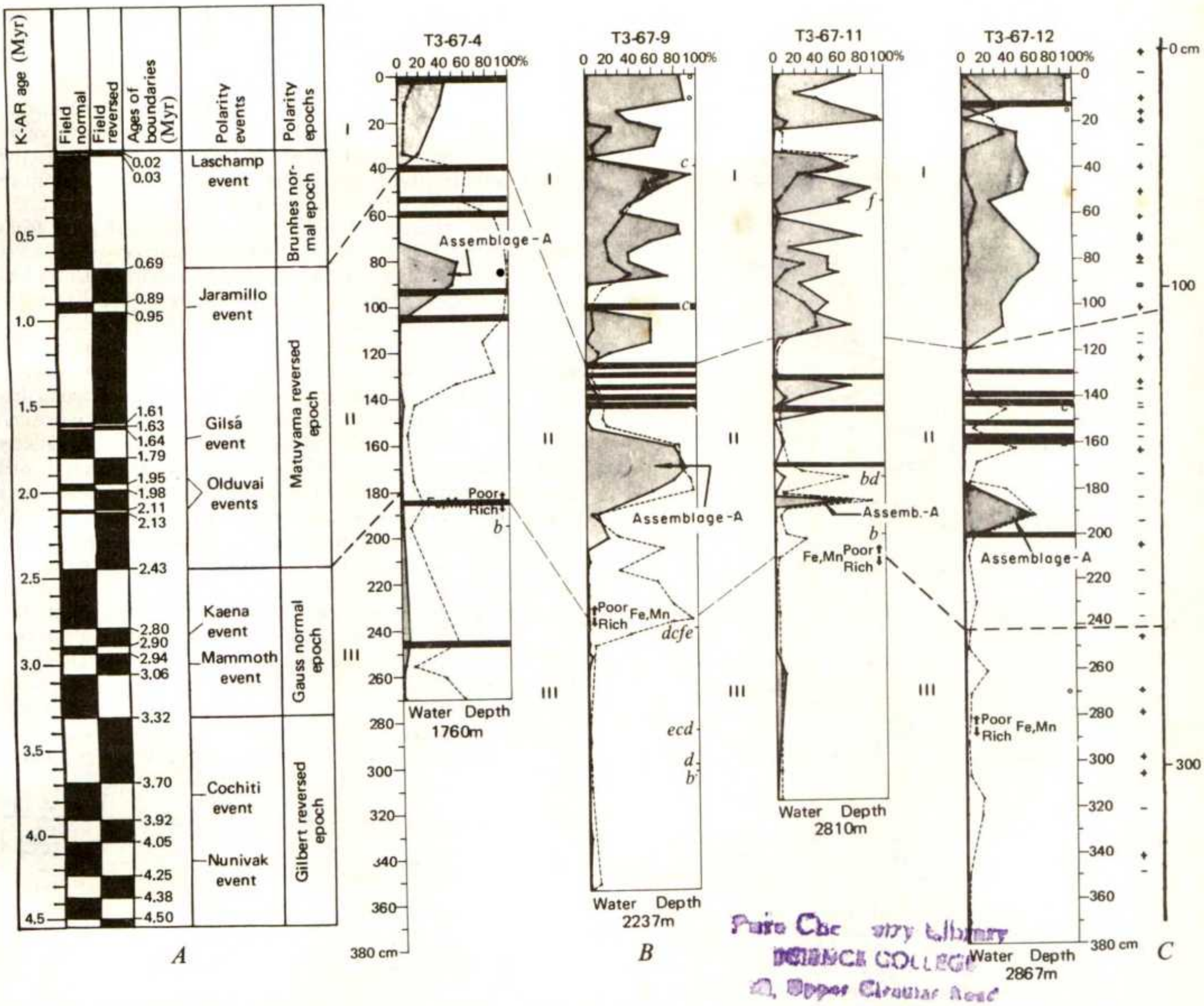

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Fig. 2 *A*, Palaeomagnetic time scale after ref. 15. *B*, Ratio of microfauna to clastics in the coarse fraction of cores T3/67-4, 9, 11 and 12. Shaded areas, microfauna, white areas, clastics. Occurrence of low latitude foraminifera: *a*, *Globorotalia menardii–tumida; b, Globorotalia scitula; c, Globorotalia* sp.; *d, Globigerinoides* sp.; *e, Globigerinoides ruber; f, Sphaeroidinella dehiscens*. Circles; Pteropods; Dark horizontal lines; zones in which benthonic foraminifera are in excess of 10% (assemblage *B*); dashed line, percentage of *G. quinqueloba* complex (including *G. exumbilicata*) out of the total planktonic foraminiferal population. *C*, Magnetic polarity changes in core T3/67-12 (ref. 16). Modified after ref. 3.

foraminifera. Two faunal assemblages, *A* and *B*, can be distinguished in this unit (Fig. 2).

In assemblage *A*, planktonic foraminifera constitute > 50% of the foraminiferal fauna. The relative abundance of *Globigerina exumbilicata* and *Globigerina quinqueloba* is high in shallow cores (< 2,000 m) and decreases considerably in deeper cores (Fig. 2), suggesting that their low frequency is attributable to solution. Data on their present-day distributional patterns and $^{18}O/^{16}O$-determinations indicate that both *G. quinqueloba* and *G. exumbilicata* are eurytherm and euryhaline species[3,5]. Low surface water salinities are believed to have determined the dominance of these two species during the Matuyama epoch. *Globigerina pachyderma* was not able to proliferate in water of low salinity, whereas *G. quinqueloba* and *G. exumbilicata* adapted to the new environment more readily. Occasionally low latitude foraminifera were observed[3,5]; assemblage *A* was deposited during mild intervals of moderate to low salinites. In assemblage *B*, benthonic deep water, calcareous species, frequently accompanied by shallow water *Elphidium spp*., constitute up to 97% of the foraminiferal fauna[5]. The dominant planktonic taxa in these zones are *G. quinqueloba* and *G. exumbilicata* (Fig. 2). Dextral *G. pachyderma* and related forms attain highest frequencies in Matuyama sediments (ref. 3, Fig. 3). Assemblage *B* was probably deposited during warmer episodes than assemblage *A*. Increased air temperatures enhanced glacier and shelf-ice melting into the Arctic Ocean and caused a further decrease in surface water salinities, leading to reduced planktonic productivity and a consequent dominance of benthonic foraminifera.

The climatic boundary between units I and II, defined by faunal and lithologic changes, corresponds approximately to the last major polarity reversal of the Earth's magnetic field, marking the Brunhes–Matuyama boundary (Fig. 2)[3,5]. The sediments of Units I were laid down over the past 0.7 Myr, during the Brunhes normal polarity epoch, a time of conspicuous climatic fluctuations, as indicated by temporal variations in the faunal composition and in the fauna–mineral ratio (refs 3 and 5; Figs. 2–4). Foraminifera-rich and foraminifera-poor beds alternate with one another, with the former representing conditions similar to those prevailing today (permanent ice cap) and containing cold water, sinistral *G. pachyderma* and related forms almost exclusively. But *G. quinqueloba* and *G. exumbilicata* attain high frequencies at the onset and end of some of these cold periods (refs 3 and 5; Figs 2 and 4), and the percentage of dextral, warm-water tolerant, *G. pachyderma* is generally highest in those zones (Fig. 3)[5]. This suggests a temporary warming with a subsequent increased supply of river and glacial melt water, followed by

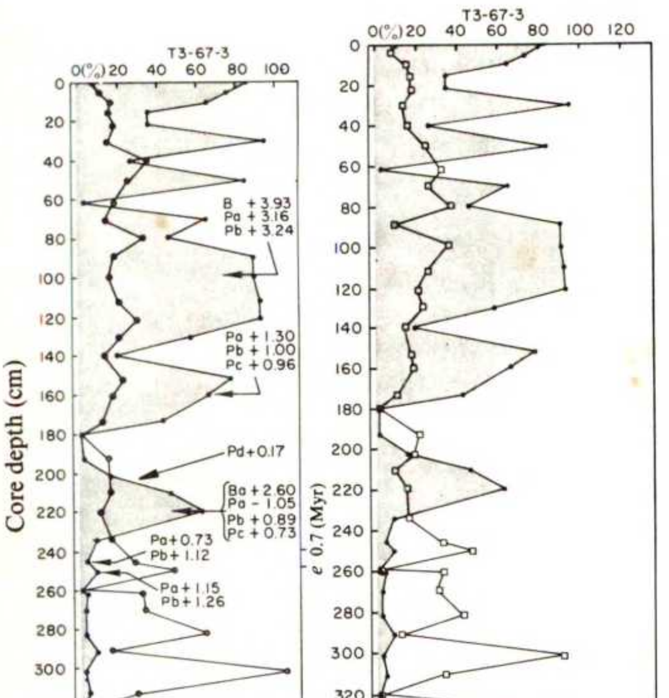

Fig. 3 Core T3/67-3. *A*, Ratio of microfauna to clastics in the coarse fraction (> 62 μm). Shaded areas, microfauna, white areas, clastics; values indicated on the abscissa at top. Occurrence of low latitude foraminifera: *e*, *Globigerinoides ruber*. Circles, percentage of dextral *G. pachyderma*; values indicated on abscissa at bottom. *B*, Same legend as for *A*. Squares, percentage of dextral *G. occlusa*. δ^{18}O values: B, benthonic foraminifera; Ba, benthonic foraminifera *C. wüellerstorfi*; Pa, *G. occlusa*; Pb, *G. pachyderma*; Pc, *G. pachyderma* (5 chambered); Pd, *G. quinqueloba*. Modified after ref. 3.

the formation of low-salinity surface water to which euryhaline–eurytherm *G. exumbilicata* and *G. quinqueloba* were able to adapt best[3,5]. In the foraminifera-poor beds which we believe represent periods free, at least seasonally, of packice, the dominant, sinistral, *G. pachyderma* and related forms are accompanied by *G. quinqueloba* and *G. exumbilicata*[3,5]. Occasionally, low latitude foraminifera occur[3,5].

Among the factors conditioning and modifying surface water salinity in the Arctic Ocean, precipitation, river runoff, evaporation, inflow and subsequent mixing of Atlantic and Pacific water, convective mixing, and the presence of driftice are considered important. The oxygen isotopic composition of Arctic Ocean surface water is determined by the same factors; evaporation results in an increase of ^{18}O whereas precipitation and river inflow causes its decrease[6]. At the present time the ranges of the temperatures and salinities of surface water are −1.6–0 °C and < 29–~34‰, respectively[7,8]. As a result of slow vertical mixing, present-day surface salinities are about 4.8‰ lower than those of the bottom water[9]. Oxygen isotope analysis of Arctic Ocean water indicates that the ^{18}O/^{16}O ratio closely follows the salinity profile, with δ^{18}O decreasing by 0.8‰ with a 1‰ decrease in salinity in the upper 350 m (ref. 9). The δ^{18}O value of this surface seawater is about 4‰ lower than at greater depth, where the δ^{18}O approaches values close to 0.

The ^{18}O/^{16}O ratio in the carbonate tests of planktonic foraminifera has been widely used to calculate past sea-surface temperatures and glacial ice volumes. The δ^{18}O values of calcareous tests depend on both temperature and the isotopic composition of the ocean. We estimated the palaeotemperatures by using the known correspondence between the coiling direction of *G. pachyderma* and present-day temperature zones in the world's oceans[1–3]. With these temperature estimates and assuming a δ value of −30‰ for glacial melt water, we calculated the palaeosalinities using the oxygen isotope palaeotemperature expression of Epstein *et al.*[4] (Table 1).

We have measured the δ^{18}O of foraminiferal tests from representative foraminifera-rich and foraminifera-poor layers in five cores (Table 1) (for results based on earlier determinations see ref. 3). The foraminifera were hand picked, treated with commercial Clorox solution to remove organic contaminants and then reacted with 100% H_3PO_4 according to the method of McCrea[10]. The liberated CO_2 was analysed for carbon and oxygen isotope ratios mass spectrometrically. We found that between the depths of 250 and 160 cm in core T3/67-3, deposited during the late Matuyama and early Brunhes (a time of relatively mild climates when the Arctic is thought to have been at least seasonally ice free, and freshwater dilution of the surface layer would have brought about density stratification), the δ^{18}O values of planktonic foraminifera differ significantly from those of the benthonic forms (ref. 3, Table 1; Figs 3 and 4). The density stratification would have been maintained for as long as the freshwater supply prevented sinking of the light, low salinity, surface water[3,5]. On the other hand, during the cold pulses of the Brunhes epoch, represented by the foraminifera-rich zones, when the presence of permanent packice increased the salinity and thus the density of the surface layers, the δ^{18}O values of the surface water approached the values of the deep water (ref. 3; 98–101 cm level in T3/67-3, Table 1; Figs 3 and 4).

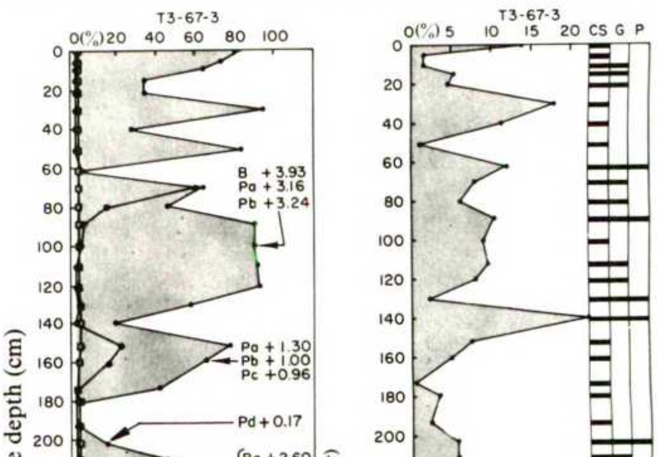

Fig. 4 Core T3/67-3. *A*, Ratio of microfauna to clastics in the coarse fraction (> 62 μm). Shaded areas, microfauna; white areas, clastics; open circles, percentage of *G. quinqueloba* of the total adult planktonic foraminiferal population; open squares; percentage of *G. exumbilicata* of the total adult planktonic foraminiferal population. *B*, Percentage of coarse fraction (> 62 μm). CS, coarse sand; G, granules; P, pebbles. δ^{18}O values: B, benthonic foraminifera; Ba, benthonic foraminifera *C. wüellerstorfi*; Pa, *G. occulsa*; Pb, *G. pachyderma*; Pc, *G. pachyderma* (5 chambered); Pd, *G. quinqueloba*. Modified after ref. 3.

Our data, together with the results of other investigators [3,5,11–14], suggest that the Matuyama epoch was a time of equable and higher global temperatures than the preceding late Gauss and the following early Brunhes[5,11–14]. As long as large volumes of glacier ice calved and melted into the Arctic Ocean during the worldwide climatic amelioration, the water did not warm up appreciably. The main result of cold, fresh water being discharged into the Arctic Ocean was the freshening of surface water. Consequently, faunal changes probably reflect primarily the effects of salinity oscillations rather than temperature changes. It is assumed that during most of the Matuyama epoch the Arctic Ocean was free at least seasonally of permanent packice, so that ice laden with debris drifted unimpeded across the ocean, melting and releasing to the sea floor the incorporated detritus. The amplitudes of global temperature fluctuations were larger during the Brunhes epoch than in the Matuyama epoch[3,5,12–14]. In the Arctic, these cold–warm pulses are expressed by the alternations of foraminifera-rich and foraminifera-poor zones, which may correspond to the mid-latitude, major, glacial–interglacial cycles.

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Electrical properties of dry and humid sand

ALTHOUGH heterogeneous, sand behaves in many ways like a typical inorganic solid. Its natural abundance makes its electrical properties important in radio propagation and in electromagnetic geological and hydrological surveys, for example using the wave-tilt technique[1]. We have undertaken an experimental study of the complex permittivity (ε', ε'') or conductivity of various natural sands at laboratory temperature in controlled humidities, over the frequency range 0.1 to 5×10^6 Hz ($T = 20 \pm 1$ °C).

A nickel-plated brass three-terminal cylindrical cell was used, with internal diameter 3.0 cm, external diameter 4.0 cm, length 4.12 cm, guard ring length 2.0 cm, guard ring spacing 0.5 mm. It was mounted in a vacuum system of ultimate pressure $\sim 10^{-2}$ mmHg, and measurements were made for several days until they did not alter, it being then assumed that the sand specimen was completely dry. Preheating *in vacuo* did not change the data. Measurements were then made with the specimen quickly transferred to a controlled humidity oven, where it was exposed either to silica gel or to saturated solutions of inorganic salts[5]. Further measurements on the dry specimens were taken in the vacuum system itself, and agree with those taken at zero humidity in the oven. The computer-controlled frequency response analyser system of Chelsea College was used at $< 10^4$ Hz; in the range 10–10^5 Hz the General Radio 1621 precision capacitance measurement system was used, and in the range 10^5–5×10^6 Hz the Wayne Kerr bridge and system SR 268 were used. There were no significant systematic discrepancies in the regions of overlap.

Table 1 Limiting permittivities, relaxation times and spreading parameters from conventional analysis

Humidity %	ε_s	ε_∞	τ (s)	α
94	1.65×10^5	3.94	2.56	0.54
74	1.36×10^4	3.16	11.1	0.46
33	240	2.73	90.9	0.42

The overall accuracy is ±0.1% in ε' and ε'', but the state of packing of the sand is less repeatable (±1.0%).

Sand specimens were taken from desert regions of Persia and Libya, and from the seashore in Derrynane, Ireland, and Sennen Cove, England; all were thoroughly washed before measurement, and sieved where necessary. The differences between data for different sands are reasonably small ($\leqslant 10\%$). Figure 1*a* and *b* represents the data for Libyan sand, which are typical.

To our knowledge, in none of the previous electrical experiments on sand has absorbed water been shown to be absent:

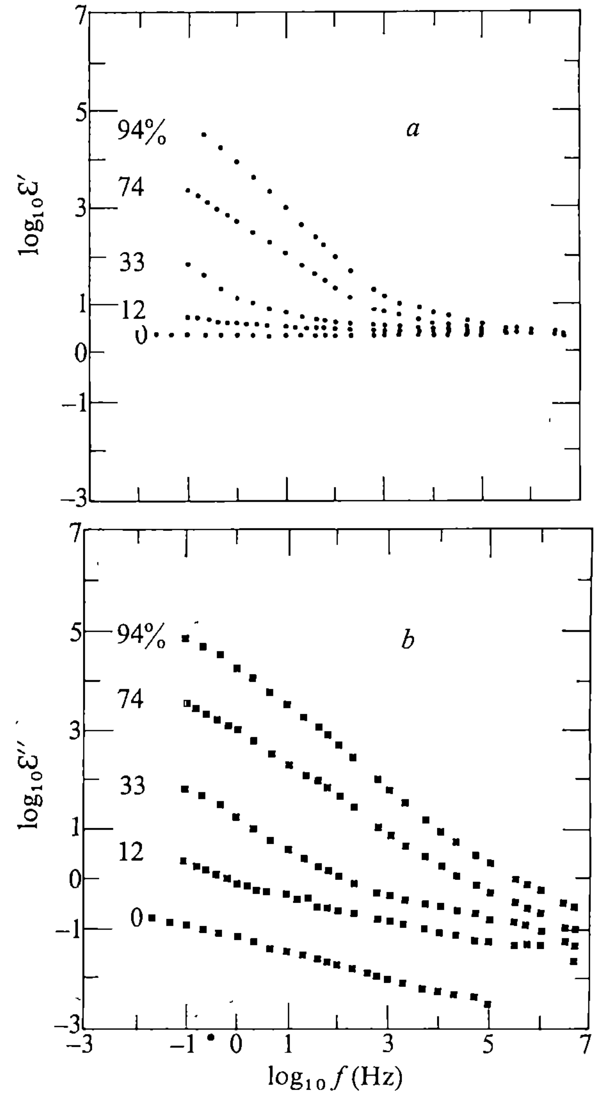

Fig. 1 *a* and *b*, Complex permittivities ε', ε'' of Libyan sand, as a function of frequency *f*, plotted logarithmically at various percentage humidities, as indicated.

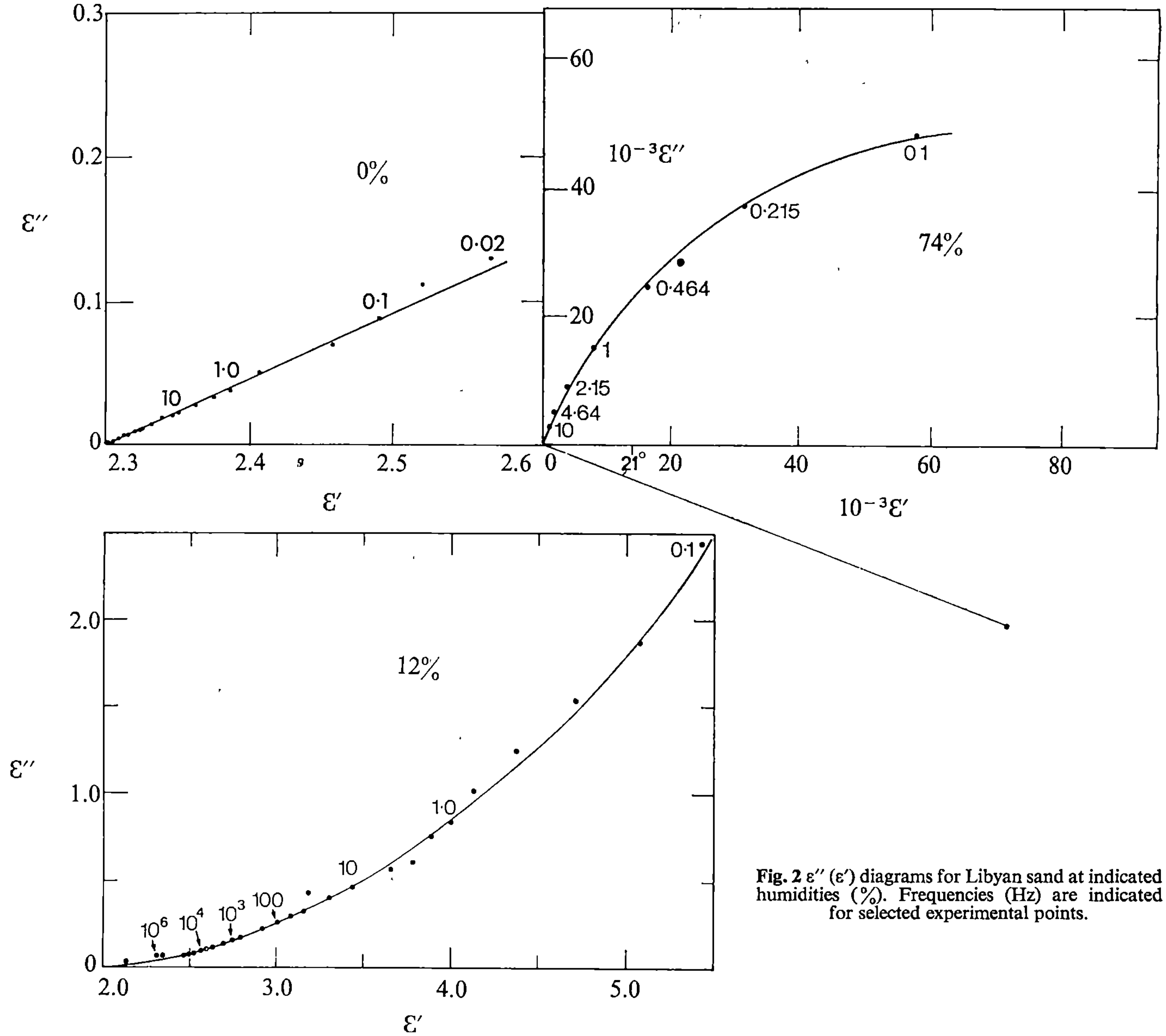

Fig. 2 ε'' (ε') diagrams for Libyan sand at indicated humidities (%). Frequencies (Hz) are indicated for selected experimental points.

laboratory exposure of the specimen after heating and vacuum drying markedly alters the electrical properties within minutes. The dry sand behaves in the manner noted and interpreted by Jonscher[2] as being common to many bulk dielectrics, in that both $\varepsilon' - \varepsilon_\infty \propto \omega^{n-1}$ and $\varepsilon'' \propto \omega^{n-1}$, where in the present instance $n = 0.77$, and ω is the pulsatance. This behaviour is characteristic of 'screened hopping' or jumping of either charge or dipoles, with the screening charge continually adjusting to the time-averaged occupancies of the sites.

Sand is a heterogeneous mixture of crystalline silica and air, and one might therefore expect dielectric mixture equations[3] to be applicable. The situation for dry sand will be qualitatively the same as that for bulk natural quartz.

For the humid sand, however, real permittivites as much as five orders of magnitude larger are found at low frequencies, which arise from the highly conducting surfaces of the grains. The specimen conducts electricity in the limit of low frequency; the d.c. conductivity is determined by drawing a horizontal tangent to the conductivity–frequency plot. This conduction corresponds to paths right across the specimen, which must be distinguished from conduction paths on grains which are insulated from each other. When the d.c. conductivity is subtracted from the data, the frequency dependence of the loss ε'' shows a broad maximum.

In the complex (ε', ε'') plane (Fig. 2, humidity 74%) the characteristic depressed semicircular arc is displayed. Conventional analysis leads to principal relaxation times τ_0, Cole and Cole[6] spread parameters α and limiting permittivities ε_s, ε_∞, as shown in Table 1. The proportion of water by weight in this sand has been determined to be 2.34% at 94% humidity.

It is difficult to reconcile such a large spread of relaxation times, with a distribution of shape factors in the Maxwell–Wagner process; we are therefore forced to conclude that there is a distribution of activation energies for surface conduction. This situation, for the counterions surrounding colloidal particles, has been treated by Schwarz[4] who took into account the potential distribution produced on the spherical surfaces by diffusion and electrostatics. His treatment did not, however, include the more modern dielectric mixtures theories which are now available[7]. Analysis of the data with their aid is in hand.

The very large low frequency variation of permittivity presents difficult problems for the interpretation of radio propagation and electromagnetic hydrological and geological survey; it is expected that clays, soils and porous rocks will behave similarly, and experimental studies are being undertaken.

The dry sand demonstrates a completely different form of ε'' (ε') diagram (Fig. 2, 0%). Because of the proportionality of the energy loss per cycle to the energy stored per cycle[2], $(\varepsilon' - \varepsilon_\infty)/\varepsilon''$ is frequency independent, and a straight line ε'' (ε') is found. At low humidities the ω^{n-1} component is raised (Fig. 1*b*) and the high frequency ε'' (ε') straight line form is enhanced by the semicircular arc which would dominate at extremely low frequencies; the resulting form (Fig. 2, 12%) displays an upward curvature. Thus the shape of the diagram indicates the relative importance of the two polarisation mechanisms proposed. At high humidities (Fig. 2, 74%) a fully developed circular arc appears. The possible contribution of variation in *n* to this situation is not yet evaluated.

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Optical resolution by chromatography at low temperature

We report here the first successful optical resolution experiments using low temperature chromatography, for which we used the tris-acetylacetonato (acac) complexes of Al(III) and Fe(III). Since the apparently unsuccessful attempts to resolve Fe-(oxalate)$^{3-}$ at −30 °C on starch[1], only room temperature experiments have been reported (for example, refs 2–4).

Using a high-pressure pump (20 MPa) the eluent 4:1 isopentane–ethyl ether, was used here, but 2 chlorobutane (or dimethyl ether could be used down to 140 K) was run (0.5–2 ml m^{-1}) through the column (diameter 7.5 mm, length 300 mm) enclosed in a themostated Cu-block in a liquid N_2 cryostat. The sample (typically 0.05 ml of a saturated ethanol solution) was injected through a septum into the eluent line just outside the cryostat. The eluate was led from the column through an optical cell and then out of the cryostat. All optical windows were birefringence free.

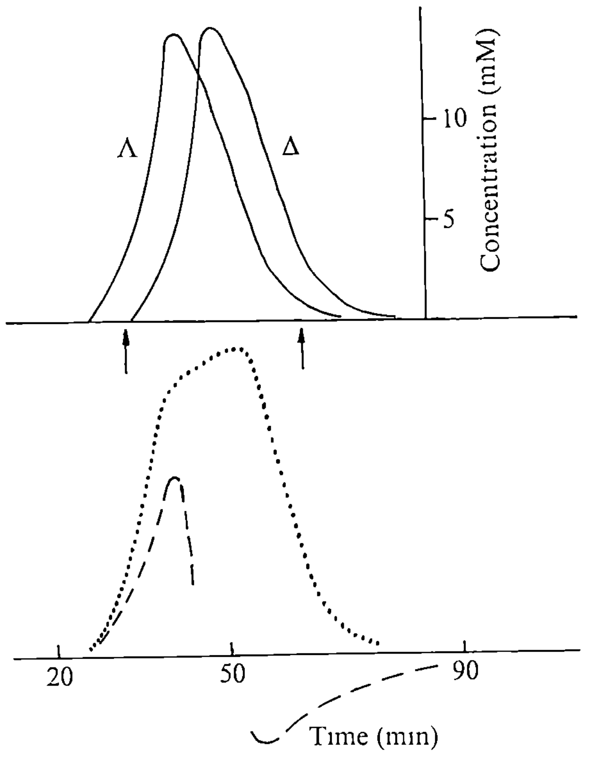

Fig. 1 A typical eluation pattern,) Al(acac)$_3$. Absorbance (···) and circular dichroism (- - -) at 303 nm against the retention time and possible corresponding concentrations of the enantiomers.

The column filling was D-lactose and Al_2O_3: a saturated solution of 4.5 g D-lactose was stirred with 10 g chromatographic Al_2O_3 (basic, 100–150 mesh). About 50% of the water was evaporated at 80 °C, and the slurry obtained was mixed with 100 ml benzene. The liquid phase was removed with a glass filter, and the filtrate was dried with air at 70 °C. Aggregation of the Al_2O_3 particles thus seemed to have been prevented.

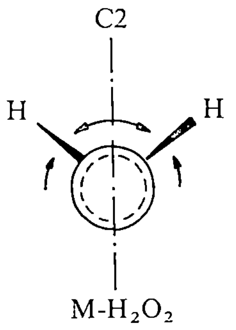

Fig. 2 The M-H_2O_2 molecule.

A high optical yield was suggested by an almost constant ratio between the circular dichroism (CD) and the absorbance (*A*) of the early fractions after the breakthrough and in some cases also by a shoulder on the absorbance curve. Fe(acac)$_3$ and Al(acac)$_3$ could be resolved below −100 °C and −30 °C, respectively. By analogy with other M(acac)$_3$ complexes on D-lactose, it is concluded that the enantiomer with the absolute configuration Λ (IUPAC nomenclature[5]) appears first from the column. The observed CD of the Fe complex was fairly diffuse (extremum at about 270 nm: CD/*A* = +1.4×10^{-4}) while Al(acac)$_3$ showed the two expected bands at 300 nm due to dipole couplings between locally excited transitions in the acac ligands: $\Delta\varepsilon$/M^{-1} cm^{-1} (the wavelength of extremum in nm, assuming perfect optical purity, is in parentheses): −54 (276), +115 (303). The spectral details and the inversion kinetics of Al(acac)$_3$ are to be discussed elsewhere[6].

The use of optical activity methods would be vastly expanded if molecules could be resolved which are (statistically) inactive at room temperature but have asymmetric ground state configurations. Hydrogen peroxide (H_2O_2), for example, with O–O axis rotation barriers which are symmetric about the C_2–O–O plane (see Fig. 2), should be a mixture of equal amounts of the two optical isomers M and P (for nomenclature see ref. 7) at low temperatures. Preliminary studies on its analogue S_2I_2 indicate that it may be resolved at 77 K by photolysis by circularly polarised radiation, as has been shown already for 1,2-dithiane[8]. With only four atoms this is the simplest conceivable optically active species.

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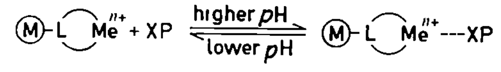
Metal chelate affinity chromatography, a new approach to protein fractionation

CONVENTIONAL nonspecific precipitation methods sometimes depend on affinities which can be used in a more selective fashion by modern chromatographic techniques. The affinity of proteins for heavy metal ions, for example, may provide a basis for their purification and analysis. A highly flexible method based on such affinities is described here.

Histidine and cysteine are known to form rather stable complexes with zinc and copper ions in nearly neutral aqueous solutions[1]. A gel loaded with strongly fixed Zn^{2+} and Cu^{2+} ions with excess binding capacity may therefore interact with surface-exposed imidazole and thiol groups of proteins. Consequently zinc and copper-containing hydrophilic gels might be selective adsorbents for histidine and cysteine-containing peptides and proteins. The same should be true of gels and ions of transition elements such as Cd, Hg, Co and Ni, which can all form coordination compounds with histidine and cysteine. To test the validity of this idea, chelate-forming ligands for these metal ions were attached to highly permeable crosslinked agarose.

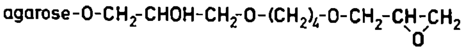
With new types of agarose derivatives[2] it is easy to synthesise chelate-forming adsorbents for proteins. If the metal is a transition element, the affinity will be *p*H dependent in such a way that selectivity for histidine, and presumably cysteine, will be favoured at the lower range (*p*H 6–8), where adsorption of proteins occurs. At alkaline *p*H, coordination with amino groups takes place, making the adsorption more effective but less selective.

To facilitate easy regeneration of the adsorbent for chromatographic purposes, metal ions must have a much higher affinity for the gel than for the substance to be separated. The principle is thus as follows



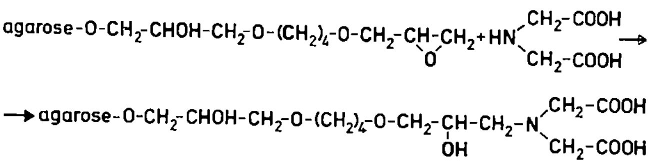
where M signifies the gel-forming matrix, L the chelate-forming ligand, Me^{n+} the metal ion, and X a metal affinity substituent in the protein or peptide XP.

For illustration we have selected serum proteins as XP, oxirane-activated agarose



$$\text{agarose-O-CH}_2\text{-CHOH-CH}_2\text{-O-(CH}_2)_4\text{-O-CH}_2\text{-}\underset{\diagdown\,\text{O}\,\diagup}{\text{CH-CH}_2}$$

as M, *bis*-carboxymethyl amino groups as L, and Zn^{2+} and Cu^{2+} as Me^{n+}. The chelate gel is synthesised according to the following scheme



$$\text{agarose-O-CH}_2\text{-CHOH-CH}_2\text{-O-(CH}_2)_4\text{-O-CH}_2\text{-}\underset{\diagdown\,\text{O}\,\diagup}{\text{CH-CH}_2} + \text{HN}\begin{matrix}\diagup\text{CH}_2\text{-COOH}\\ \diagdown\text{CH}_2\text{-COOH}\end{matrix} \longrightarrow$$

$$\longrightarrow \text{agarose-O-CH}_2\text{-CHOH-CH}_2\text{-O-(CH}_2)_4\text{-O-CH}_2\text{-}\underset{\text{OH}}{\text{CH}}\text{-CH}_2\text{-N}\begin{matrix}\diagup\text{CH}_2\text{-COOH}\\ \diagdown\text{CH}_2\text{-COOH}\end{matrix}$$

Disodium salt of iminodiacetic acid (2 g) was dissolved in 10 ml of 2 M Na_2CO_3 and added to 15 ml of suction-dried oxirane-activated agarose (Sepharose 4B) in a conical flask. The suspension was heated to 65 °C and stirred for 24 h. The gel was collected on a fritted-glass filter and washed with distilled water. The product, *bis*-carboxymethyl amino agarose (see reaction scheme above), contained 630 µmol of *bis*-carboxymethyl amino groups per g dry gel (25 µmol per ml swollen gel) as determined by nitrogen analysis according to the Kjeldahl method and titration of carboxyl groups.

Gel-bound chelates of zinc and copper were prepared by passing water solutions of zinc chloride ($ZnCl_2$, 1 mg ml^{-1}) and copper sulphate ($CuSO_4.5H_2O$, 1 mg ml^{-1}) through columns (1×3.6 cm) containing the *bis*-carboxymethyl amino agarose. The Zn^{2+} column was completely saturated with Zn^{2+}, as shown by the presence of Zn^{2+} in the eluate, whereas the Cu^{2+} column was loaded with Cu^{2+} only to 60% of its length to avoid contamination of the eluate with Cu^{2+}. The chelate gel was able to adsorb about 20 µmol of Cu^{2+} per ml.

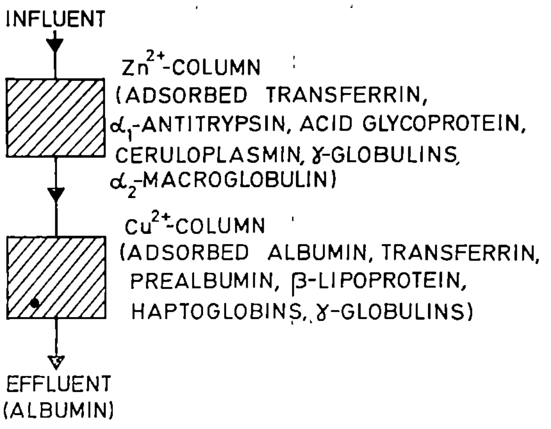

INFLUENT
Zn²⁺-COLUMN
(ADSORBED TRANSFERRIN, α₁-ANTITRYPSIN, ACID GLYCOPROTEIN, CERULOPLASMIN, γ-GLOBULINS, α₂-MACROGLOBULIN)
Cu²⁺-COLUMN
(ADSORBED ALBUMIN, TRANSFERRIN, PREALBUMIN, β-LIPOPROTEIN, HAPTOGLOBINS, γ-GLOBULINS)
EFFLUENT
(ALBUMIN)

Fig. 1 Coupling of composite column.

Human serum (2.7 ml) was fractionated by passing it through the two columns coupled in sequence, beginning with the Zn^{2+} column (Fig. 1); the two columns were then disconnected and each was eluted separately (Fig. 2). Neither Cu^{2+} nor Zn^{2+} was detected in the eluate until, in the final elution step, EDTA solution was passed through the columns (Fig. 2).

The fractions obtained were subjected to electrophoresis on gradient acrylamide gel plates (4–30%) in a Gradipore 342000 apparatus. A sample of 30 µl of the material with A_{280} of 1–3 was applied to the top of the plate and electrophoresis was run for 24 h in 0.089 M Tris–borate buffer (*p*H 8.3) containing 2.5 mM EDTA. The separated proteins on the gel plate were stained with amido black and destained with acetic acid (7%). The components tentatively identified are summarised in Table 1 (numbers indicate their relative order of appearance in the fraction).

The fractionation seems to be very sharp for many of the compounds and no signs of denaturation were observed. All electrophoretic bands in the original serum were detected in

Fig. 2 Composite chromatogram obtained by elution of the coupled Zn^{2+} and Cu^{2+} columns. (*a*) The two columns were washed with 0.05 M Tris-HCl buffer, *p*H 8, 0.15 M in NaCl (60 ml each). The adsorbed material was removed from each column separately, according to the following scheme Cu^{2+} column (*b*): 1, 0.1 M Na phosphate buffer, *p*H 6.5, 18 ml; 2, 0.1 M Na phosphate buffer, 0.8 M in NaCl, *p*H 6.5, 20 ml; 3, 0.1 M Na acetate buffer, 0.8 M in NaCl, *p*H 4.5, 17 ml; 4, 0.05 M EDTA, 0.5 M in NaCl, *p*H 7.0, 25 ml. Zn^{2+} column (*c*): 5, 0.1 M Na phosphate buffer, *p*H 6.5, 47 ml; 6, 0.1 M Na phosphate buffer, 0.8 M in NaCl, *p*H 6.5, 38 ml; 7, 0.1 M Na acetate buffer, 0.8 M in NaCl, *p*H 4.5, 12 ml, 8, 0.05 M EDTA, 0.5 M in NaCl, *p*H 7.0 10 ml.

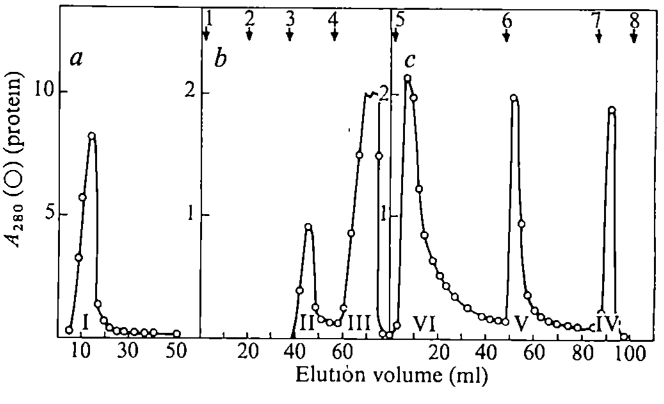


Table 1 Components identified by Gradipore electrophoresis of the fractions obtained in metal chelate affinity chromatography of human serum on Cu^{2+} column and Zn^{2+} column

	Peak	Components identified
Material that passed both columns in conditions of adsorption: 0.05 M Tris-HCl, *p*H 8, 0.15 M NaCl	I	Albumin
Material desorbed from Cu^{2+} column: Na acetate, 0.8 M NaCl, *p*H 4.5	II	Albumin, γ-globulins, prealbumin, traces of α_1-antitrypsin
0.05 N EDTA, 0.5 M NaCl, *p*H 7.0	III	Albumin, transferrin, haptoglobins, β-lipoprotein, traces of γ-globulins
Material desorbed from Zn^{2+} column: Na phosphate, *p*H 6.5	IV	Transferrin, α_1-antitrypsin, acid glycoprotein, γ-globulins, ceruloplasmin
Na phosphate, 0.8 M NaCl, *p*H 6.5	V	Transferrin, traces of haemoglobin*, traces of γ-globulin
Na acetate, 0.8 M NaCl, *p*H 4 5	VI	α_2-Macroglobulin, traces of haemoglobin*

*Present as contaminant in the serum used.

the fractions and no new bands were observed. In the same starting buffer, but in the absence of heavy metal ions, very little protein was adsorbed. At 1 M NaCl the metal chelate gel was still an efficient adsorbent. The involvement of ordinary ionic adsorption in the fractionation is therefore ruled out.

Orienting experiments have shown that of the amino acids present in proteins, histidine and cysteine are most strongly adsorbed. Histidylhistidine is very strongly adsorbed at *p*H 7 as a consequence of the cooperative effect of the histidine residues. Other amino acids are also adsorbed at *p*H 8–9 but are desorbed far in advance of histidine when the *p*H is lowered. We therefore believe the chromatographic behaviour of a protein to be largely governed by its surface number or density of exposed imidazole and thiol groups. In addition, other groups such as indole may be important.

With the metal chelates we have tested, the adsorption capacity for serum proteins decreased in the following order: Cu > Zn > Ni > Mn, with Cu being very effective and the Mn gel having almost negligible adsorption capacity. There also seem to be qualitative differences that are worth exploring.

Metal affinity has been used to purify proteins containing or dependent on metal ions[3]; however, the method described here is more general in its application and not restricted to proteins. The potential advantages and limitations of this method will have to be explored further, but the capacity and separation power seem to make it suitable for large scale fractionations.

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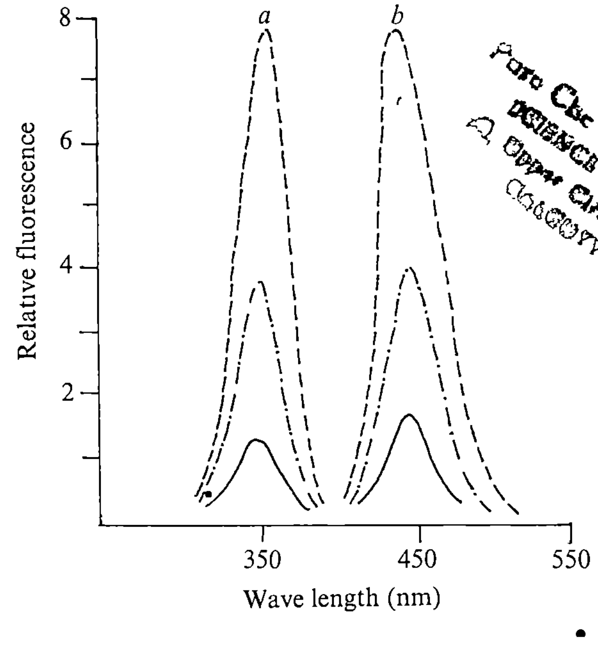
Fluorescent product accumulation in ripening fruit

STUDIES of mammalian tissue indicate that free radicals mediate fluorescent product accumulation[1–3] and that such products are causatively related to cellular senescence[4–7]. Fluorescent products isolated from senescent tissues, cells stressed by oxidants such as ozone, or cell-membrane isolates have characteristic fluorescence at 360–380-nm excitation and 440–470-nm emission[8]. These pigments have been identified in various mammalian tissues[1–3], protozoa[11], nematodes[12], and insects[6,13,14]. We are not aware of any reports of the occurrence of lipofuscin-like pigments in plant tissues. This study reveals the presence, in ripening fruit, of fluorescent material of spectral characteristics identical to lipofuscin from mammals. The pigment is present at greater concentration in a subcellular fraction than in the whole tissue and has a molecular weight in excess of 4,000. The yield of fluorescent product increases with ripening of banana and pear fruits.

Fluorescent products were extracted from the peel and pulp tissue of ripening banana (*Musa cavendishii,* var. Valery) and pear (*Pyrus communis,* var. Bartlett) by the procedure described by Fletcher *et al* [15]. Lipofuscin isolates were water-washed and irradiated with ultraviolet light to remove possible interfering substances[15]. A crude mitochondrial fraction was isolated from banana pulp by the procedure outlined elsewhere[16]. Molecular weight was estimated with a 58×2.5 cm column packed with Sephadex LH-20 in chloroform–hexane (65 : 35, v/v) The eluant was monitored for fluorescence with a flow cell at 360-nm excitation and 440-nm emission. Fluorescence spectra were obtained with an Aminco–Bowman spectrophotofluorometer at 10 °C with 3-, 1- and 3-mm slits for the 3-, 4- and 6-slit positions. The machine was standardised with 0.1 μg ml^{-1} quinine sulphate in 0.1 N H_2SO_4. The standard

Fig. 1 *a*, Excitation and *b*, emission spectra of chloroform-soluble lipofuscin isolated from ripening Bartlett pear fruit. – – –, Over-ripe; – · – · –, ripe; ——, green fruit.



had a fluorescence intensity of 1.9 units at 360-nm excitation and 440-nm emission. The relative fluorescence units were derived from the following formula: (Meter multiplier value/100) × % fluorescence. The fluorometer blank was adjusted and solvent subtracted.

Excitation and emission scans of lipofuscin isolates from preclimacteric, climacteric and postclimacteric pear are illustrated in Fig. 1. These spectra are similar to those obtained from banana fruit and reported for mammalian sources of fluorescent product[17]. Yield of the fluorescent substance from banana pulp increased exponentially after treatment of green fruit with 100 p.p.m. ethylene (Fig. 2). A linear increase in fluorescent pigment occurred during peel ripening. Similar increases in pigment also occurred during ripening of pear fruits (Fig. 1).

The mitochondrial fraction isolated from postclimacteric pulp contained approximately sevenfold more lipofuscin than did the whole pulp tissue. The emission spectrum of mitochondrial lipofuscin was broader (peaking at 450 nm) than that for the whole tissue isolates. Banana mitochondria lipofuscin had spectra similar to those reported by Chio and Tappel for *in vitro* oxidised rat liver mitochondria[4]. Fluorescence yield from heavier and lighter sedimented cell fractions was similar to the whole tissue. The higher yield of fluorescence from the mitochondrial fraction may reflect the occurrence of lysosome-like particles containing membrane debris as indicated in electron microscopic analyses of ripening fruit[18].

The fluorescent substance isolated from banana pulp eluted in the void volume of a Sephadex LH-20 column indicating a molecular weight in excess of 4,000.

These data support our contention that a lipofuscin type of pigment accumulates during ripening and proceeding senescence of fruit. The excitation and emission spectra of fruit lipofuscin are comparable with those reported for animal lipofuscin The pigment is barely detectable in preclimacteric fruit and increases with ripening and postclimacteric development. The association of this pigment with a particulate fraction and the high molecular weight are also consonant with their lipofuscin identity. Our suggestion that peroxidative damage to cell membranes is a primary event of plant senescence is supported by other experiments. Cellular membranes from plant tissues contain relatively large amounts of polyunsaturated fatty acids in their amphipathic lipids. Ripening of fruit is associated with a decline in the highly unsaturated fatty acids of membrane lipids[19,20]; increased activity of various oxidative enzymes[21–23] and gross changes in membrane integrity[24,25]

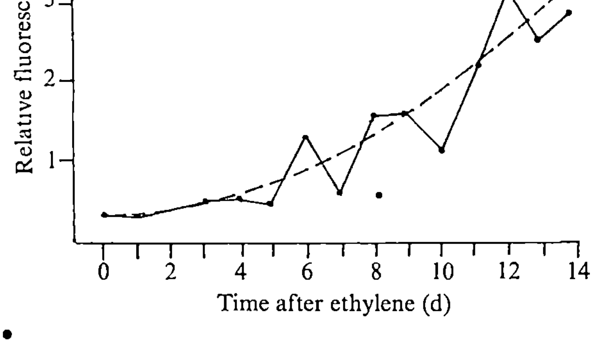


Fig. 2 Recovery of fluorescent products (chloroform soluble) from ripening banana fruit after treatment with ethylene. Day zero fruit were number 2 (full green pulp), day 5 were number 4 (yellow flush pulp), day 8 fruit were number 5 (full yellow with green tips and day 14 were number 8 (brown spotted pulp). Pulp tissue (5 g) was extracted with 20 ml of chloroform–methanol solvent (2:1, v/v). Data are representative of two additional trials. A similar increase in lipofuscin was also observed in the peel tissue. Excitation wavelength was 360 nm and emission wavelength was 440 nm.

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Lectins stimulate pollen germination

Pollen germination involves the conversion of the quiescent vegetative cell into a metabolically active and rapidly growing pollen tube[1] Although many external factors influence pollen germination, no predominant control mechanism has been identified. In certain lymphocytes in culture, lectins (plant agglutinins) have been shown to stimulate quiescent cells to enlarge and synthesise DNA[2,3]. Lectins also bind to the surface of actively growing cells and cause agglutination of red blood cells and transformed or malignant cells[2–4], of gametes of *Chlamydomonas*[5] and of carrot protoplasts[6]. There have, however, been few studies of the effects of lectins on plant tissues. The results presented here show that the lectins, concanavalin A (con A) and phytohaemagglutinin-M (PHA), stimulate pollen of *Lilium longiflorum* to germinate more rapidly than controls, by reducing the length of the lag period before pollen tube emergence.

Mature pollen from greenhouse-grown plants of *Lilium longiflorum* (cv Ace) was collected from anthers dehisced at room temperature. The pollen was mailed from Urbana, Illinois to Berkeley, without special precautions and stored at 4 °C over Drierite. Samples (10 mg) of pollen were germinated in 50-ml flasks containing 2 5 ml of germination medium[1] (1.27 mM $Ca(NO_3)_2 . 4H_2O$, 0.99 mM KNO_3, 0.16 mM H_3BO_3, and 0 29 M sucrose) in a shaker water bath at 30 °C. Con A (Sigma: Grade IV, highly purified) and PHA (Calbiochem: B grade) were dissolved in the germination medium. In some experiments weighed pollen samples were humidified before germination in a closed container over water for 1 h (ref. 7). This procedure improved germination, especially in pollen which had been stored for one month or more. Aliquots of pollen were taken from the flasks at hourly intervals and the germinated grains were identified and counted microscopically.

In solution of 1 mg ml^{-1} con A, the largest percentage of pollen grains germinated during the first hour in the germination medium, while that of control pollen grains germinated during the third hour (Fig. 1). In 100 μg ml^{-1} con A, the maximum germination rate occurred during the

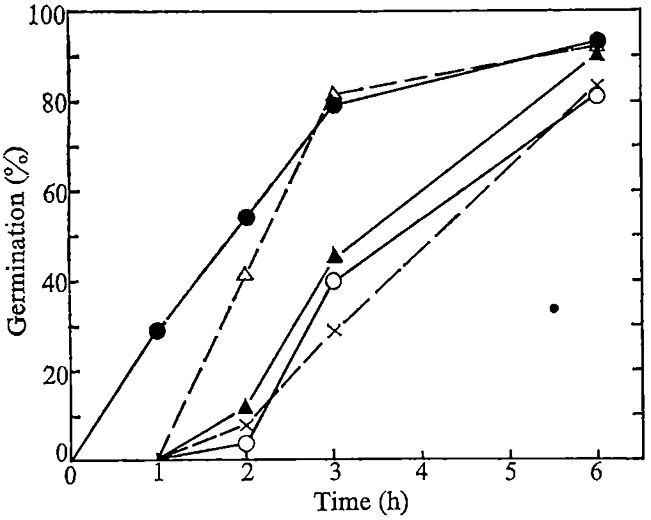


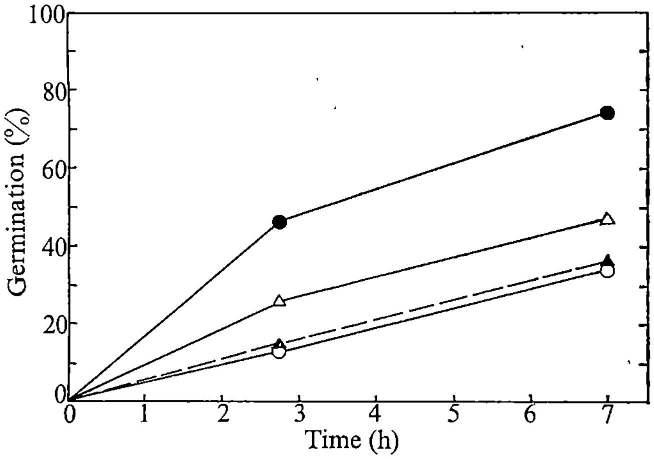
Fig. 1 Percentage germination of pollen, one week old, humidified, with con A in the germination medium. ●, 1 mg ml^{-1} con A; △, 100 µg ml^{-1} con A; ▲, 10 µg ml^{-1} con A; ×, 1 µg ml^{-1} con A; ○, control.

second hour. At concentrations of 10 µg ml^{-1} con A, the germination rates were only slightly higher than the control rates and at 1 µg ml^{-1} con A, they were slightly lower than the controls. The total percentage of pollen which germinated approached the same value for the control after about 6 h as for all the con A concentrations tested. With the addition of 0.1 M α-methyl mannoside, a competitive inhibitor of con A binding, the stimulatory effect of con A was reduced by 20–50%.

In older pollen, which had been stored for seven weeks, the lag period was shortened by about 2 h and the maximum percentage germination attained was doubled when con A was added to the germination medium (Fig. 2).

The stimulatory effect of PHA was greater than that of con A (Fig. 3). The greatest number of pollen grains germinated during the first hour with PHA (1 mg ml^{-1}), during the second hour with con A (1 mg ml^{-1}) and no sooner than the fourth hour in the control. Comparable experiments which ran for a longer time (up to 20 h) showed that the maximum percentage of grains eventually germinating in the control was similar to that germinating in either PHA or con A. Pollen in 100 µg ml^{-1} PHA germinated slightly faster than the control, while the difference between germination rates in 10 µg ml^{-1} PHA and in the control was not significant.

Fig. 2 Percentage germination of pollen, seven weeks old, humidified, with con A added to the germination medium. ●, 250 µg ml^{-1} con A; △, 100 µg ml^{-1} con A; ▲, 2.5 µg ml^{-1} con A; ○, control.



Since lectins did not increase the maximum percentage of grains which eventually germinate, their action may be similar to the events which occur spontaneously over a longer period. The stimulation of germination by lectins which are known to bind to cell surfaces of red blood cells, fibroblasts, lymphocytes and spermatozoa from a range of animals[3] suggests that primary control of the germination process may occur at the plasma membrane. Pollen germination is affected by sugars and ions, especially calcium, which are taken up from the external medium either *in vivo* or *in vitro*[8,9]. An alteration of the permeability properties of the plasma membrane by bound lectins could lead to greater uptake of water or nutrients for germination. Ion fluxes involving calcium have been described around germinating lily pollen and localised influxes of cations have been postulated to influence pollen tube growth[10]. Substances which bind con A have been found in the walls of pollen grains; however, they can be readily leached out of the walls and do not seem to remain bound to the cell wall or plasma membrane[11].

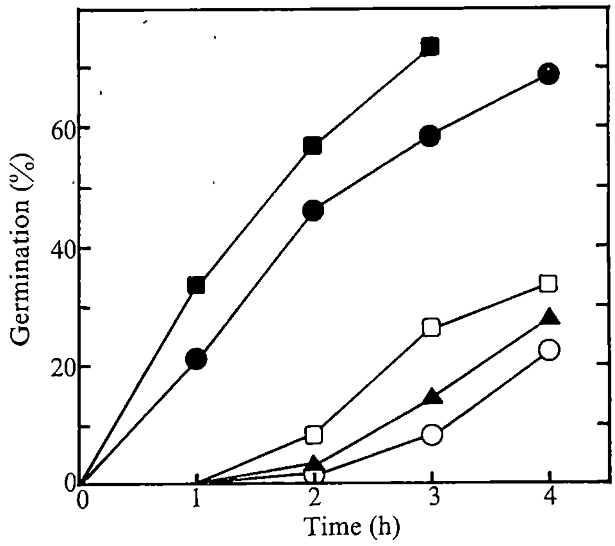


Fig. 3 Percentage germination of pollen, one week old, unhumidified, with PHA added to the germination medium. One sample was given con A. ●, 1 mg ml^{-1} con A; ■, 1 mg ml^{-1} PHA; □, 100 µg ml^{-1} PHA; ▲, 10 µg ml^{-1} PHA; ○, control.

Although con A and PHA stimulate the uptake of ^{3}H-thymidine by lymphocytes[3,12] it seems unlikely that they exert their effect on the synthetic activities of germinating pollen. Neither DNA nor RNA synthesis is required for pollen tube initiation[13].

The stimulation of pollen germination by con A and PHA as manifested by a reduction in the length of the lag period is similar to the stimulatory effect of the stigmatic exudate on pollen germination[14]. It is therefore possible that the events mediated by lectins *in vitro* may also occur during normal germination *in vivo*.

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Adaptive radiation in the subgenus *Scaptodrosophila* of Australian *Drosophila*

UNTIL a recent survey, based on the two major museum collections of Australia[1], the Australian *Drosophila* fauna was poorly known and few endemic species had been described. This survey covered 81 species including 40 described as new. The world total of *Drosophila* species is known to exceed 1,250 (ref. 2); the Australian fauna thus represents about 6% of this total. By comparison with the world *Drosophila* fauna, the Australian total contains a disproportionately large number of species (45 out of 81) in the subgenus *Scaptodrosophila*, most of them endemic although a few are also known from neighbouring South-east Asian and/or Pacific regions. The Australian *Drosophila* fauna is therefore unique in that nowhere else have members of the subgenus *Scaptodrosophila* so dominated the *Drosophila* fauna of a large region.

The three other major subgenera are poorly represented. The eleven known Australian *Hirtodrosophila* species are all endemic to Australia, but in the subgenera *Drosophila* and *Sophophora*, each of which contains more than 500 species worldwide[3], four species only of *Sophophora* are endemic to Australia; these subgenera are represented in Australia principally by the cosmopolitan species, although a few of the South-east Asian/New Guinean species extend into northern Australia.

Of the *Scaptodrosophila* species, only three are endemic to south-western Australia; a further two occur in both east and west, and the remainder have southern and/or northern distributions in the eastern part of the continent only[1]. Laboratory culture of most species has proved very difficult, complicated by the almost complete lack of information on ecology and life histories; there are some indications that the larvae of at least some species may be leaf miners pupating in soil. Equally, phylogenetic lineages within the sugbenus are substantially unknown[1]. The Australian *Scaptodrosophila* radiation does not, of course, compare with the famous speciation in the subgenus *Drosophila* in Hawaii[4], in which small area more than 350 species have already been described—about four times the total number of known Australian species in the genus *Drosophila* and seven times the number of known Australian *Scaptodrosophila;* but the Hawaiian proliferation is a special case unmatched in any other comparably small region of the world.

The major *Scaptodrosophila* radiation in Australia has thus occurred in the eastern part of the continent, and within this region many of the species seem to be restricted to the southern part of the country (especially Victoria and New South Wales). One of the more common species is *D. inornata* Malloch, which is frequently closely associated with tree ferns (*Dicksonia* spp.)[5]. The most favourable habitats for *Drosophila* species in south-eastern Australia seem to be wet sclerophyll/rain forest areas characterised by tree ferns and various other ferns. All habitats, including a few sheltered habitats without tree ferns, have permanent water, and often a deep layer of rotting leaf litter[6]. Most of the *Scaptodrosophila* species are not attracted to baits and so far can only be collected by intensive sweeping. Extensive spring and summer collections have recently been made in habitats of the above type in Victoria and the east coast of southern New South Wales (Fig. 1). More than 2,000 *Scaptodrosophila* representing at least twelve species, at least two of them new, were collected in damp habitats. Locality records of the museum collections[1] reveal the likelihood that most of the remaining known species are confined to damp habitats. It seems likely in view of the number of sites at which collections are still to be made that further species await discovery; in any case species once regarded as extremely rare are now known to be relatively common and widespread.

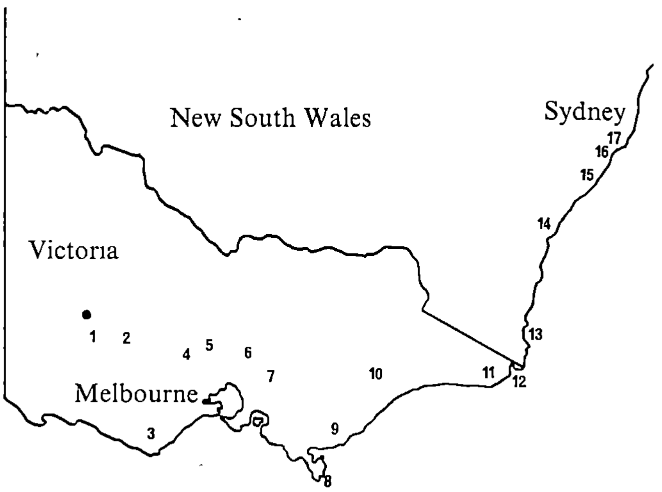

Fig. 1 Map of Victoria and south-eastern New South Wales indicating continental islands where endemic *Drosophila* spp. have been found.

The damp habitats most favourable to *Scaptodrosophila* species are normally isolated from one another, often by considerable distances. Localities of major habitats sampled so far are indicated in Fig. 1. The temperature/humidity requirements of the flies are such that migration among many of the habitats in summer, especially in Victoria, must be impossible[6]. This is because Victoria has a hot dry summer during which the habitats are damp, cool refuges, offering temperature–humidity conditions compatible with survival of *Drosophila* species, but outside these refuges the environmental stress would be excessive. (Similar environmental constraints occur for the cosmopolitan species[7].) There is, therefore, the possibility of studying the evolutionary biology of *Scaptodrosophila* species in essentially continental islands which have probably been separated for at least 6,000 yr, that is after the end of an era of high precipitation, high temperature and high rainfall[8].

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Autonomous pigment movement in the radial pupil of locust ocelli

ALTHOUGH pigment movement is important for modulating luminous intensity on the invertebrate retina, its mechanisms and control cannot be investigated easily since the cells in question are largely inaccessible to experimental interference and often observable only by electron microscopy. The preparation described here is directly observable and is also potentially amenable to manipulation.

The ocellus of the mature adult locust is a simple lens eye comprising a lens up to 500 µm diameter overlying a transparent vitreous layer and retina, behind which is a neuropile and ocellar nerve[1]. The whole cup-shaped structure is invested by a densely pigmented epithelial layer continuous with the epidermal cells outside the ocellus and a ring of specialised epidermal cells lying between the vitreous layer and the lens. The cells of this ring adjacent to the lens are clear corneagenous cells. The more proximal of these cells contain numerous reddish-brown pigment granules which in *Schistocerca* are confined to the periphery of the ocellus and thought to be stationary[1]. My observations of an Indonesian acridid, *Valanga nigricornis* and an Australian species, *Austracorus guttulosa* indicate that this pigment is moveable in some acridids.

Light-adapted ocelli show a distinct pupil lying in the middle of the eye, on dark adaptation the pupil expands so that the eye presents a pure white fundus with only a margin of pigment visible (Fig. 1*a*). Pigment movement has no apparent circadian rhythm and is controlled only by light falling on that ocellus, illumination of the compound eyes or other ocelli being ineffective. The fully contracted pupil may be a circular aperture or a vertical slit. The Indonesian species examined showed circular pupils in lateral and median ocelli whereas *Austracorus* showed slits on the lateral ocelli and a round pupil on the median. The degree of pigment movement varies widely between individuals and may differ considerably between the right and left ocelli of a single animal. Close inspection of ocelli under a dissecting microscope revealed an optical discontinuity in the centre of the lens which proved, in the scanning electron microscope, to be the result of a circular or oval ridge on the back surface (Fig. 1*b*). While pigment in the extreme light-adapted condition of some ocelli did not extend as far as this ridge, pigment was never found to intrude within this circle, and in many cases the pupillary margin was defined by this ridge (for example, Fig. 1*a*). Light and transmission electron microscopy show the central region of the lens to be free from pigment and corneagenous cells, the vitreous cells here abutting the lens directly. The crest of the ridge is also punctuated with holes of about 2 µm diameter, which in life are presumably occupied by processes from the vitreous cells to form a stockade delineating the inner margin of the pigment cells.

The efficiency of the pupil as a mechanism for reducing the light intensity at the retina cannot be calculated readily unless it can be shown that the field of view of the ocellus is not significantly influenced by pupil diameter, direct measurements of field size show that this condition is satisfied (unpublished observations). As Fig. 2*a* shows, the area reduction in the pupil is of the same order of magnitude as the light reflex in the human eye[2], the time course on the other hand is orders of magnitude slower. The response–intensity function for pigment cells has been quantified by observation of pupil diameter through a Leitz incident illumination microscope used with Ultrapak objectives. Incident light passing through a narrow-band interference filter (546 nm peak) and attenuated with neutral density filters was allowed to fall on an ocellus of an intact but immobilised animal. Measurement of pupil diameter was made in red light (628 nm) after 90 min adaptation to the test intensity. The dynamic range of pigment movement extends for only 2.5 log units, by visual standards a narrow range (Fig. 2*b*). In comparable conditions the human pupil has a dynamic range of 7 log units (ref. 2).

In view of this narrow dynamic range the control mechanism for pigment movement is particularly interesting. Some simple experiments were therefore carried out to explore the obvious proposition that pigment movement requires the neural elements of the ocellus.

Once the head capsule is opened, gentle pulling on the ocellar nerve will detach the vitreous from the pigment ring distal to it. The lens and its adhering pigment iris may then be isolated and observed *in vitro*. Unfortunately the pigment cells do not survive well in the usual locust Ringer solutions[3,4]. Immersion in silicone oil is less injurious, and in favourable preparations reversible pigment movement in response to light can be elicited for several hours after excision. The trauma of surgery, however, is a frequent cause of failure. A much more reliable preparation is obtained by allowing the pigment to remain in the haemolymph in the absence of the neural elements of the

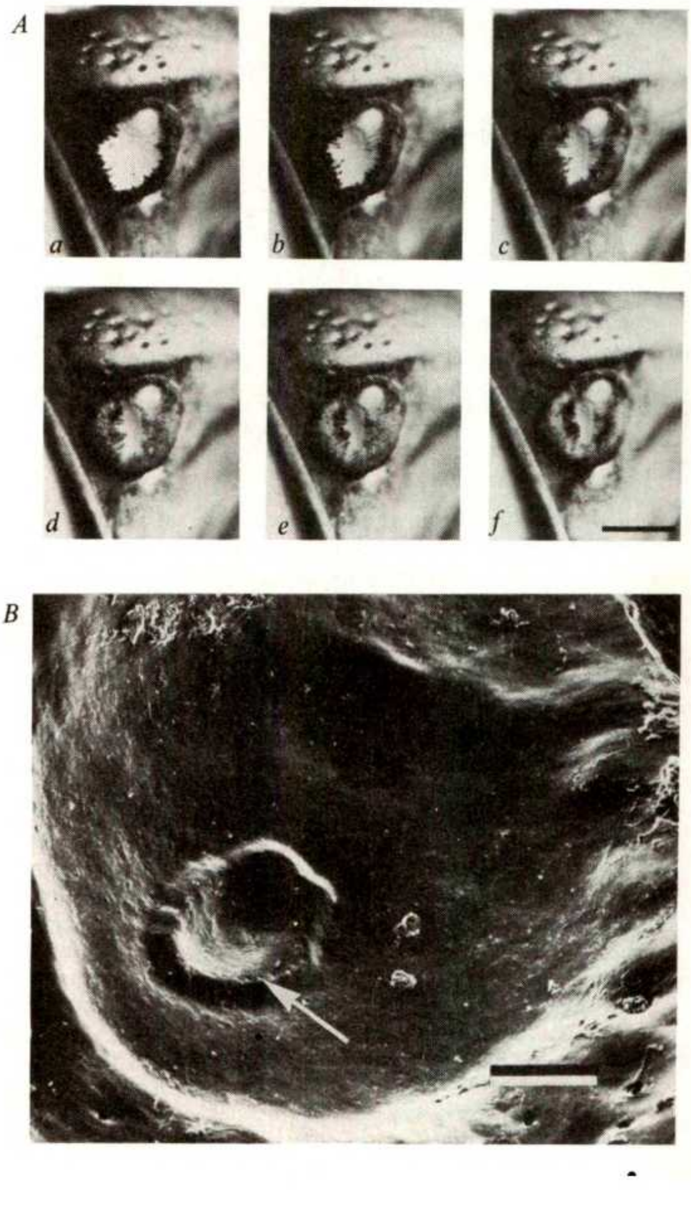

Fig. 1 *A*, Contraction of the pupil in the right lateral ocellus of *Austracorus* after the onset of an intense white light. The fully contracted pupil is clearly a vertical slit in these ocelli, the optical discontinuity caused by the ridge on the back of the lens may just be discerned in (*b*) and (*c*). Times after stimulus onset were: *a*, 0 min; *b*, 3 min; *c*, 6 min; *d*, 9 min; *e*, 15 min; *f*, 40 min. The bar represents 500 µm. *B*, Scanning electron micrograph of the back surface of a lateral ocellus from an Indonesian acridid. The whole lens is a shallow concavity in the centre of which is a pronounced circular ridge. The crest of the ridge is punctuated with 2-µm diameter holes, indicated by the arrow, the bar represents 100 µm.

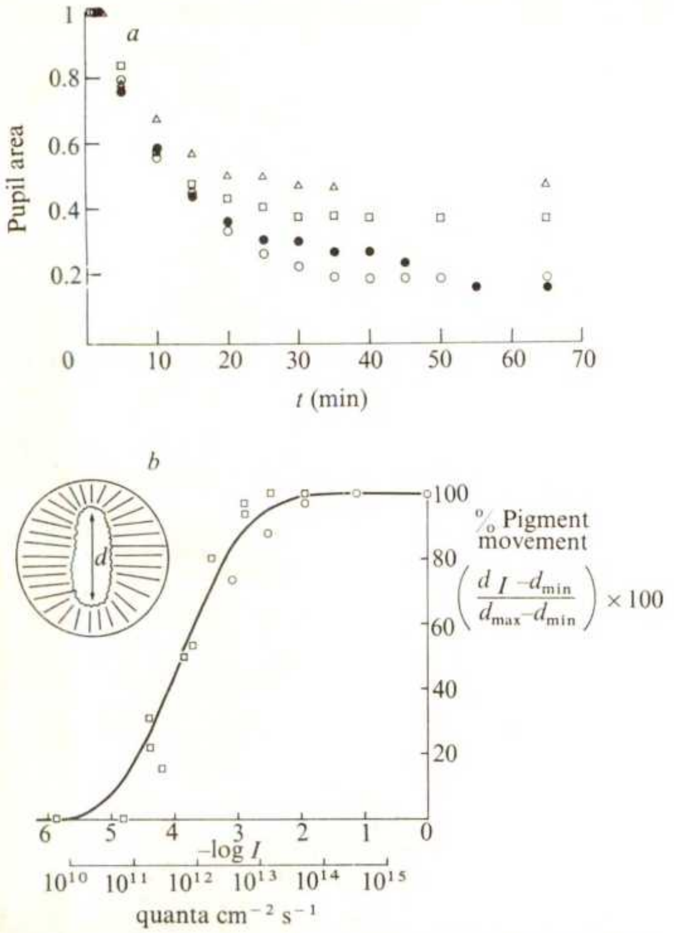

Fig. 2 *a*, Reduction in pupil area as a function of time after intense stimulus onset. The results from four lateral ocelli of *Valanga* normalised relative to their initial (dark adapted) pupil areas, maximum apparent pupil area was about 0.25 mm^2. Making reasonable assumptions about the positions of the principal planes of the thick lens in these ocelli the real pupil is unlikely to be more than 15% smaller than the apparent magnified pupil. *b*, Pigment movement as a function of intensity of monochromatic green light for a lateral ocellus of *Austracorus*. □, Long axis of the pupil; ○, short axis. Quanta $\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$ were determined by substituting a calibrated PINIO diode for the ocellus. Since the incident illumination was not perpendicular to either ocellus or detector but was a hollow cone of light with the object at the apex these figures are only approximations.

ocellus. A small hole in the exoskeleton dorsal to an ocellus allows the introduction of a tungsten wire hook with which the ocellar bulb may be extracted from the head capsule. A cut then serves to remove the ocellus and a length of ocellar nerve. If the hole is resealed the pigment remaining attached to the lens is viable for several days, responding to light as it did before surgery.

From the simple experiments reported here it is concluded that pigment movement in the ocellar iris is truly autonomous, being controlled by neither neural nor hormonal mechanisms but by processes intrinsic to the pigment cells. Although nothing is known about the method of transduction of light by these pigments cells, the recent reports of transduction by pigment grains within *Aplysia* central neurones suggest an obvious parallel[5,6]. The significance of the pupil in the functioning of the ocellus is presumably as a mechanism for modulating the intensity of light at the retina, for the other two possibilities—increased depth of focus[7] and reduction in field of view (unpublished observations)—have been eliminated. Although the maximum reduction of intensity achieved by the pupil is a meagre 0.7 log units it is likely that, as in the case of the human pupil[8], even a modest effect is worthwhile.

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Effects of hyperthermia on rat embryos in culture

TEMPORARY elevation of the body temperature, resulting from climatic influences or infection, occurs in many mammals. Such hyperthermia is often harmless, but in the pregnant female it may be teratogenic, with particularly damaging effects on the brain of the developing embryo. The studies that have been made on experimentally induced hyperthermia and teratogenesis in animals are reviewed in ref. 1 and suggest that hyperthermia may also be teratogenic in man. The methods used to raise the temperature in experimental animals have included incubation of the whole animal[2], injection of foreign protein[3], short-wave diathermy[4], immersion of the uterus in heated saline[5,6], and microwave radiation of the uterus[7]. In most of these experiments the period of exposure to high temperature (often only 40–60 min) was limited by the tolerance of the animal and the need to avoid excessive resorption of the embryos. The temperature experienced by the embryos usually varied during treatment and could not be controlled precisely.

When the embryos are explanted and grown in culture they may be exposed to raised temperatures for longer and in closely specified conditions. It also becomes possible to distinguish direct effects of hyperthermia from those that may result indirectly from changes in the maternal metabolism. We report here some results obtained from a study on rat embryos explanted during early organogenesis (a stage of development known to be particularly sensitive to teratogenic agents) and grown in culture in various conditions of hyperthermia.

Rat embryos of CFHB strain were explanted on the tenth day of pregnancy and grown in culture for 46–47 h in rotating glass-stoppered bottles by the method of New *et al.*[8]. The culture medium was pure rat serum, containing 50 μg ml^{-1} streptomycin, equilibrated with a gas mixture of 5% O_2, 5% CO_2, 90% N_2 which was changed to 20% O_2, 5% CO_2, 75% N_2 after the first 24–27 h of culture. The culture bottles were rotated in purpose-built incubators with an overall temperature accuracy of ±0.2 °C. In earlier experiments, the control incubator was set at 37 °C, but this was later raised to 38 °C to conform more closely with measurements of deep rectal temperature of rats at mid-pregnancy. No difference was found in development at 37 or at 38 °C.

At explantation, the embryos were 'egg cylinders', 1–2 mm long. In most, the earliest stages of neural fold development were visible but no further organogenesis had occurred.

Table 1 Development of embryos grown in culture during the temperature schedules shown in Fig. 1

		Total no. of embryos	Blood circ.	No. of embryos showing: Turning completed	Fused allan.	Normal organ.	Final protein (µg mean ± s.e.)
Series *A*	*a*	27	23	18	22	27	130 ± 12
	b	27	16	20	17	13	127 ± 10
	c	27	0	3	3	2	52 ± 6
Series *B*	*a*	12	9	11	12	12	127 ± 10
	b	12	1	4	12	9	65 ± 5
	c	12	0	0	12	0	43 ± 3
Series *C*	*a*	18	11	14	14	15	176 ± 9
	b	18	3	11	8	5	134 ± 9
	c	18	15	17	13	15	146 ± 10
Series *D*	*a*	41	40	38	37	39	121 ± 6
	b	41	32	25	34	31	101 ± 4
	c	41	33	21	32	25	90 ± 4

Blood circ., Indicates the number of explants which showed a normal vigorous blood circulation in both embryo and yolk sac at the end of the culture period.

Turning completed., Number of embryos which had completed the rotation from the early dorsally-concave curvature to the more characteristic foetal position with the ventral surface concave.

Fused allan., Indicates that the allantois had extended from the hind end of the embryo and fused normally with the chorion.

Normal organ., Indicates that no abnormalities of organogenesis could be detected.

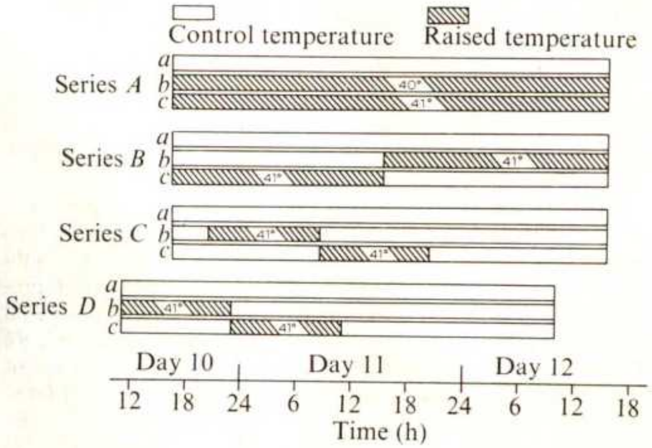
After examination, most of the embryos (without the embryonic membranes) were digested in caustic soda and assessed for total protein content by a colorimetric method[9].

At the end of the culture period, most of the embryos incubated at control temperature had formed about 23–26 somites with brain and spinal cord, optic and otic vesicles, anterior limb buds and a well-developed heart and blood circulation (Fig. 2). A yolk sac, amnion and chorioallantois were also present.

Four series of experiments were made, with temperature schedules as shown in Fig. 1. In each series, one group of embryos (*a*) was cultured at control temperature and the other two (*b*, *c*) were exposed to raised temperature for some time. To ensure that the three groups were comparable, each litter of embryos was divided equally between them. The results are summarised in Table 1.

In series *A* (81 embryos) a comparison was made of the development of embryos exposed to control temperature (37–38 °C), to 40 °C, and to 41 °C throughout the culture period. At 40 °C, overall growth was similar to that of the controls (Fig. 2) although a smaller proportion of these embryos had a good blood circulation at the end of culture, and about half showed small developmental abnormalities, including a few with slight microcephaly. In contrast, the embryos incubated at 41 °C were very severely affected. Final protein content was very significantly less than that of embryos incubated at either 37–38 °C or 40 °C ($P<0.001$). Somite formation was so poor at 41 °C that somite counts could not be made. More than half the embryos in this group were microcephalic and almost as many had enlarged hearts and oedema of the pericardium; few had begun to turn, and in 6 of the 27, the posterior part of the trunk was sharply bent back on the anterior, with fusion of the anterior and posterior neural folds.

Fig. 1 Schedules of heat treatment applied to rat embryos in culture.



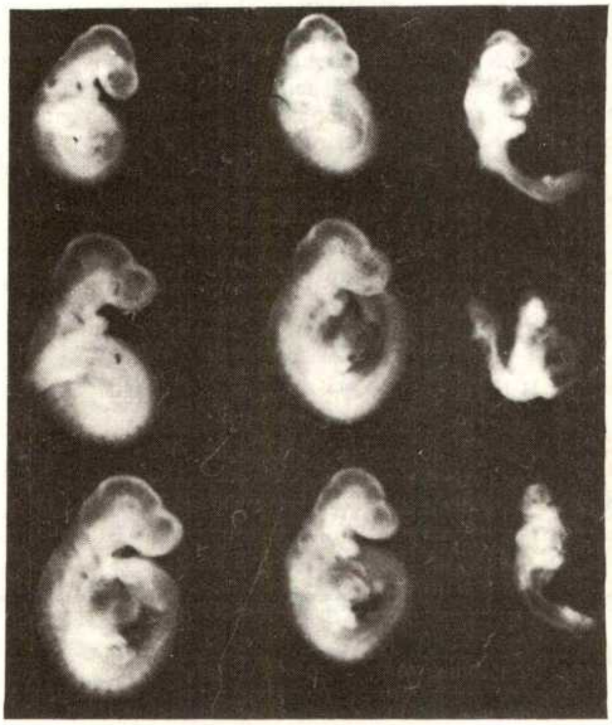


Fig. 2 Nine embryos from the same rat, explanted at early head-fold stage and grown in culture for 46 h (series *A*). Incubated at 38 °C (left), 40 °C (centre) and 41 °C (right). (The embryonic membranes have been removed.)

In series *B* (36 embryos), the duration of exposure to 41 °C was reduced to half the 46-h culture period, but this was enough to produce many abnormalities. The embryos incubated at 41 °C for the first 23 h had a very low protein content and were much smaller than the controls. The somites were badly developed. None of these embryos had turned, and none had a blood circulation at the end of culture. Half were microcephalic and nearly as many showed pericardial oedema. The embryos incubated at 41 °C for the second 23 h were better formed, though a few had turned and only one had a normal blood circulation. They were also very small, with a protein content significantly lower than that of the controls ($P<0.001$)

though significantly higher than that of the embryos exposed to 41 °C for the first half of culture ($P<0.002$).

In series *C* (54 embryos) and *D* (123 embryos), exposure to 41 °C for various 12-h periods produced relatively few gross malformations, but the protein content was always significantly lower than that of the controls. The most severely affected embryos were those in group *b* of *C* and in group *c* of *D*. Both were exposed to 41 °C during the first few hours of day 11, which may indicate that this is an exceptionally sensitive period. Common abnormalities were microcephaly and pericardial oedema. Blood circulation, rotation of the embryo, and fusion of allantois and chorion were also affected.

A notable feature of these results is the very small temperature rise needed to produce abnormalities of development. Some defects were apparent when the temperature was raised only 2 degrees above the normal for rats (that is, to 40 °C) and gross abnormalities were found in nearly all the embryos when the temperature was raised to 41 °C throughout the culture period. Such small rises are well within the range commonly experienced during fever and suggest that studies of this kind on the embryos of laboratory animals in culture may be relevant to problems of hyperthermia and teratogenesis in man. The frequent appearance of defects of the head agrees with the findings of others[2,5,6] on hyperthermia applied to embryos *in vivo* at this stage of development. Finally, it may be noted that the present study has demonstrated direct effects of hyperthermia on the development of mammalian embryos isolated from the maternal metabolism.

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Enzyme variability in the protochordate amphioxus

COMPARING protein polymorphisms in 24 species of invertebrates and 22 species of vertebrates, Selander and Kaufman[1] observed greater genetic variability on average in the invertebrate than in the vertebrate by three criteria: number of polymorphic loci (25–50% in invertebrates compared with 10–20% in vertebrates); mean heterozygosity per locus per individual (15% compared with 6%); and, average number of alleles per polymorphic locus (three compared with two). I have found that amphioxus, a fish-like protochordate, combining vertebrate characters (such as dorsal neural tube throughout life) and invertebrate characters (such as solenocytic protonephridia) resembles, in its enzyme variability, invertebrates rather than vertebrates.

I have done electrophoresis of extracts of 44 specimens of an Australian species of amphioxus, *Branchiostoma moretonensis*[2], collected in Moreton Bay, Queensland and flown to Adelaide. All individuals were surveyed both in starch gel and in acrylamide gel. Most variation was observed in both systems, although to identify isoalleles glucose-6-phosphate dehydrogenase had to be run in parallel in both systems. Bands that had been thought to be two distinct loci coding for the set of rapidly migrating glucose-6-phosphate dehydrogenases in *Branchiostoma lanceolatum*[3] represent variants with identical electrophoretic mobility of the major zone but non-identical mobility of the satellite zone. The enzymes listed in Table 1 were localised, after electrophoresis, by conventional methods[4,5].

NADP malate dehydrogenase, 6-phosphogluconate dehydrogenase, glutamate dehydrogenase and alcohol dehydrogenase, usually readily detectable after electrophoresis of extracts of tissues from most animals, could not be localised after electrophoresis of amphioxus extracts[3]. For some extracts extremely faint zones were observed after 24 h of staining for NADP isocitrate dehydrogenase and for L-lactate dehydrogenase; such low activity may represent reaction on contaminants in the staining reagents and, in any case, is likely to be unreliable in scoring for variation.

Table 1 shows that amphioxus resembles Selander and Kaufman's[1] average invertebrate rather than their average vertebrate in the amount of enzyme variability. For 10 out of 14 enzyme loci there is clear genetically interpretable[4] variation, with an average of 4.2 alleles per locus. The average amphioxus is heterozygous at 24% of these enzyme loci. Using Avise and Selander's[6] definition that a locus is polymorphic only when the commonest variant is present in an allelic frequency of 0.95 or less, nine of the 10 loci with genetically interpretable variation are polymorphic. It can be calculated that for a sample of 44 individuals, representing a sample of 88 genes for each diploid autosomal locus, the chances of detecting variation at this 0.95 cutoff point is close to $99\%–(0.95)^{88} = 0.011$.

One unusual observation is that there is a large deficit of heterozygotes for the phosphoglucomutase polymorphism: 22 homozygotes were observed compared with 9.74 expected. A binomial test[7] shows that the probability of such an extreme deviation is less than 0.001. Ayala *et al.*[8] observed a statistically significant deficit of heterozygotes for phosphoglucomutase in the clam *Tridacna maxima* but explained this observation as "our scoring is biased against heterozygous genotypes" (page 185) as a result of "allozymes with little difference in migration" (page 186).

There is no overlap between any of the six variants of phosphoglucomutase in amphioxus; thus, the very highly statistically significant deficit of heterozygotes cannot be the result of problems in electrophoretic resolution. In the survey of amphioxus no other polymorphic protein had a statistically significant deficit, or excess, of heterozygotes.

The data on amphioxus are significant from three other aspects.

First, there is partial association of variants of glucose-6-phosphate dehydrogenase and phosphoglucomutase. Individuals with the electrophoretically faster variants of phosphoglucomutase tended to have a somewhat greater frequency of the electrophoretically slower variants of glucose-6-phosphate dehydrogenase and vice versa (Table 2). The probability of such a partial association occurring by chance can be estimated by treating the data in Table 2 as a 2×2 contingency table and, after correcting for continuity, calculating the χ^2 statistic[7]; the χ^2 is 6.71 with one degree of freedom, significant at the 0.01 level. No other association of variants for different enzymes was observed. As phosphoglucomutase and glucose-6-phosphate dehydrogenase are adjacent enzymes in connecting glycolysis and the pentose pathways, this epistatic interaction may be biochemically significant.

Second, these results demonstrate variability in a 'primitive' animal. Although amphioxus has some characteristics which probably represent secondary specialisation, there is no doubt that it is a primitive form, resembling a hypothetical prevertebrate so well that "if amphioxus had not been discovered it would have to have been invented" (page 388 of ref. 9). According to J. Z. Young[10] (page 46) "amphioxus shows us approximately the condition of the early fish-like chordates, living in the Silurian some 400 million years ago, and . . . has undergone relatively little change in all the time since". My

Table 1 Enzyme polymorphism in amphioxus

Enzyme (EC number)	No. of alleles	Gene frequencies*	Hetero-zygosity (%)
Group I enzymes (components of energy yielding and major biosynthetic pathways[12])			
Hexokinase (2.7.1.1)	3	0.02; 0.83; 0 15	26
Phosphoglucomutase (2.7.5.1)	6	0.04; 0.22; 0.28; 0.15; 0 26; 0 04	50
Glucose-6-phosphate dehydrogenase (1.1.1.49)			
'Fast'	8	0.11; 0.16; 0.27; 0.02; 0.24; 0 15; 0 01, 0 03	68
'Slow'	Variable		
Glucose-phosphate isomerase (5.3.1.9)	3	0.04; 0.92; 0.04	14
NAD diaphorase (1 6.-.-)	2	0.01; 0.99	
NAD-dependent L-malate dehydrogenase (1.1.1.37)			
'Fast'	Monomorphic		
'Slow major'	2	0.07; 0.93	
Group II enzymes (relatively nonspecific: esterases, peptidases and so on[12])			
Tetrazolium oxidase	Monomorphic		
α-Naphthyl acetate esterases (3.1.-.-)			
'Fast'	8	0.08; 0.19; 0.23; 0.16; 0.19; 0.08; 0.03; 0.03	84
'Middle'	2	0.08; 0.92	
'Slow'	Monomorphic		
Phe–leu peptidases (3.4.-.-)			
'Fast'	4	0.04; 0.81; 0.13; 0.02	29
'Slow'	2	0.62; 0.38	41

*Listed in order of electrophoretic mobility, pH 8.0 starch gel; most anodal variant first.

[illegible] Library
[illegible] College
[illegible], Upper Circular Road
Calcutta

findings with amphioxus resemble those of Selander *et al.*[11], who observed a moderately large amount of variation in a survey of 16 enzymes in the xiphosuran *Limulus polyphemus*, a phylogenetic relict of at least 200 Myr. The living representatives of these two bradytelic lines are no less polymorphic, at least at the enzyme level, than representatives of rapidly evolving lineages.

Third, there is the variability in enzymes involved in energy production and major biosynthetic pathways, compared with more peripheral enzymes of often lower substrate specificity. Enzymes active in energy metabolism, "group I", have less electrophoretic variation than the so-called "nonspecific enzymes", "group II", in which have been placed esterases, phosphatases, peptidases, amylases, alcohol dehydrogenases and aldehyde oxidase[12,13]. Amphioxus has less heterozygosity for the group I enzymes (Table 1), but the difference is not statistically significant: 77 heterozygotes out of 352 opportunities for group I enzymes compared with 71 heterozygotes out of 264 opportunities for group II enzymes—$\chi^2 = 1.82$ (1 d.f.). I found sharp electrophoretic resolution for all enzymes in Table 1 except for 'slow' glucose-6-phosphate dehydrogenase. Several enzymes in the energy-yielding and major biosynthetic pathways are large molecules, often composed of several subunits. While it is reasonable to expect that such enzymes may be more conservative in their electrophoretic variability, it is also possible that some of the apparent lack of polymorphism reported in the literature is the result of limitations in electrophoretic technique where large multi-subunit enzymes often give less sharp resolution than small single-subunit proteins.

Table 2 Partial association of variants of phosphoglucomutase and glucose-6-phosphate dehydrogenase

Glucose-6-phosphate dehydrogenase	Phosphoglucomutase: The two electrophoretically faster variants (A and B)	Phosphoglucomutase: The four electrophoretically slower variants (C, D, E and F)
The four electrophoretically faster variants (A, B, C and C^A)	12 (15.5)*	53 (39)*
The four electrophoretically slower variants (D, E, E^B and F)	19 (15.5)*	25 (39)*

* The numbers in parentheses are the expected values for each category if the numbers were distributed at random.

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Non-selective isolation, stability and longevity of hybrids between normal human somatic cells

FIFTEEN years after the first somatic cell hybridisation[1], there are still no reports of the successful isolation of proliferating populations of pure synkaryons between strains of euploid mammalian cells. Migeon *et al.*[2] fused suitably marked human fibroblasts and lymphocytes using a selective medium, but they were unable to isolate further and characterise the presumptive hybrid subpopulation. No one, to our knowledge, has been able to obtain synkaryons between euploid cells without the use of biochemical selective techniques. Such an achievement would be highly significant, since it would facilitate mating between any pair of euploid somatic cells for which there are few selective[2], but many[3] differentiating markers.

In the experiments summarised in Table 1, we fused fibroblast cultures from pairs of normal male human donors, one with the A, the other with the B form of X-linked glucose-6-phosphate dehydrogenase (G6PD). The data of Table 2 were derived from fusions among cells of a skin culture from a female heterozygous for G6PD$_A$ and G6PD$_B$, some of her cells being of the A type and others of the B type. In theory, one-third of the tetraploid clones emerging from dilute platings of approximately equal cell mixtures of G6PD$_A$ and G6PD$_B$ types should be identifiable as hybrids, provided that there is no preferential fusion between like cells, no precipitous chromosomal loss or diploidisation during the initial period of clonal growth, no significant tetraploid cell population due to causes other than cell fusion, and no selective growth for hybrids, selfed tetraploids or parentals. Our results show that hybrid isolates were, in fact, obtained, even though at yields substantially below expectation. Among the mixoploid isolates listed in Tables 1 and 2, there was a total of five cultures with a distinct heteropolymer component in addition to the A and B band, but the distribution of presumptive subunits deviated from the typical hybrid (1:2:1) pattern.

The success of the procedure we describe seems to be dependent on sufficient growth potential of each of the parental strains, effective heterokaryon formation and ability to identify tetraploid colonies. We found that our previously described, relatively laborious quantitative method of ploidy determination[6] could be simplified by visual comparison of metaphases of suspected tetraploid clones with those of control diploids (the predominating type) in the same dish. With some experience, it is possible to screen and classify 200–300 colonies within one hour. We believe that most of our mixoploid isolates arose not from erroneous identification, but from contamination with diploid cells during the plating and/or isolation procedures, which obviously can be minimised by plating at lower densities and by earlier transfers of presumptive tetraploid clones (before contact between colonies). We have

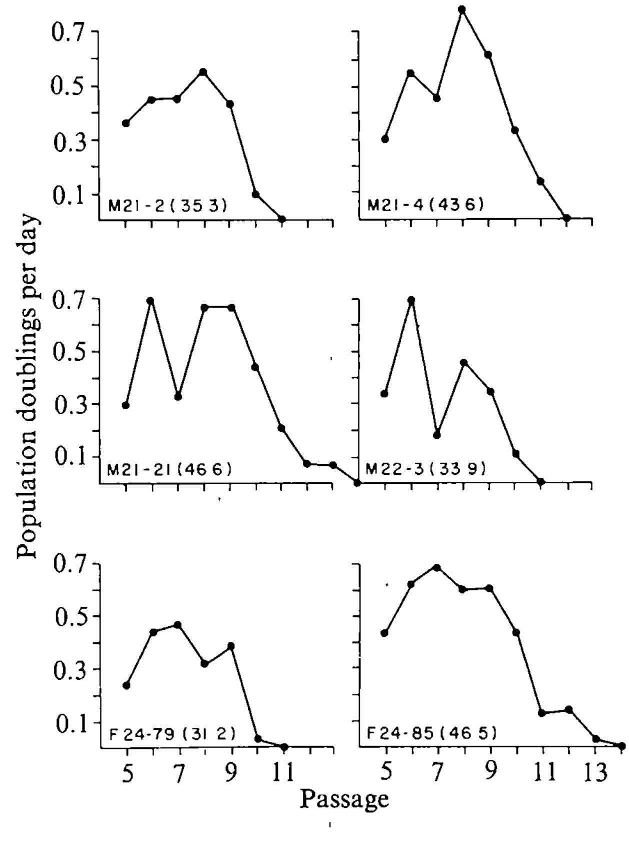
Table 1 Summary of cytogenetic and electrophoretic classification of clonal isolates from 8 separate fusion experiments involving 5 different G6PD$_A$ and G6PD$_B$ strains

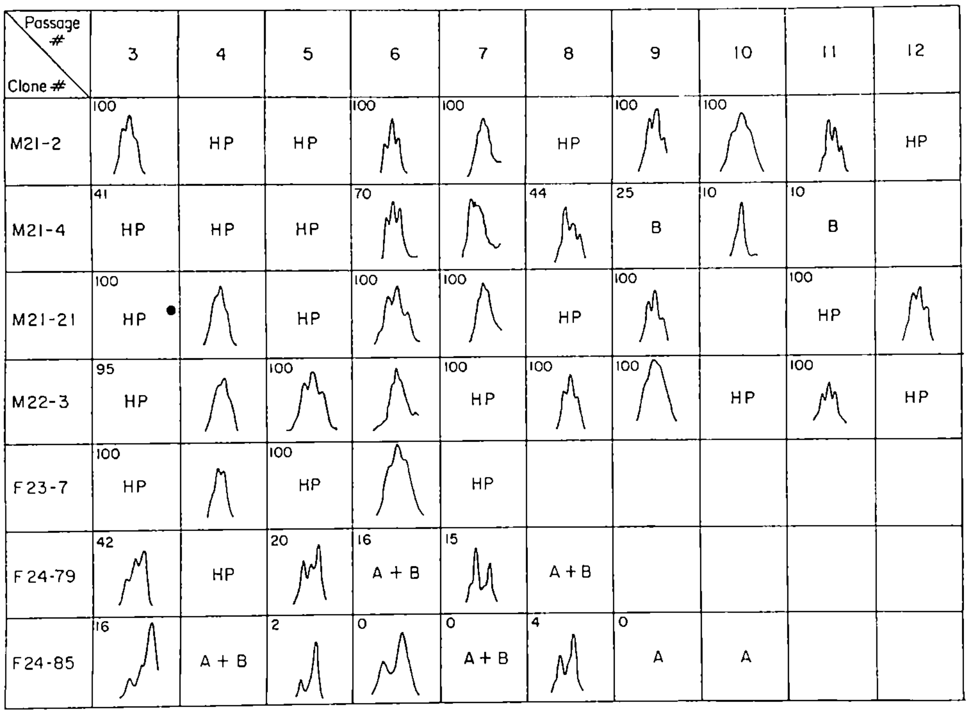
Type of fusion:	Total clones screened/isolated (3,203/108*)			
G6PD$_A$ × G6PD$_B$ (male strains)	G6PD A	G6PD B	G6PD A+ B	G6PD Heteropolymer
100% Diploid	10	15	5	
>90% Diploid	3	1	1	
Diploid + tetraploid		24	12	2
>90% Tetraploid		7		1
100% Tetraploid	3	15		3

* 6 not tested.

Fibroblast cultures were established from foreskins or skin biopsies and grown according to techniques described previously[4]. For fusion, 0.5×10^6 mitotic or interphase cells of each of the G6PD$_A$ and G6PD$_B$ strains, and 1×10^6 cells in the case of the G6PD$_{AB}$ heterozygote, were suspended in 0.5 ml of chorioallantoic fluid[5] containing inactivated Sendai virus and kept on ice. After 20 min, the virus cell suspensions were transferred to a 37 °C water bath and incubated for 60 min. The preparations were then diluted with 10 ml of complete media, centrifuged, resuspended, and plated into 2–4 60-mm dishes at a density of 9,000 cells per cm^2. Twenty-four hours later, one of the dishes was trypsinised and dilute-plated at densities of 6–8 cells per cm^2 into 100-mm Petri dishes. A typical experiment involved the preparation of 10 such dishes, which received two feedings before they were screened for the presence of tetraploid clones at days 9–11 after plating Tentative identification of tetraploid clones was based on visual comparison (Nikon inverted microscope, model MS) of the sizes of the equatorial plates[6] of metaphases of the living clones contained within a 100-mm dish. Isolation of all clones judged to be tetraploid was by a steel cylinder technique[7]. All isolates were transferred to 35-mm Petri dishes and further subdivided after 3–5 d. One dish each was used for cytogenetics, electrophoresis and further passage. For cytogenetics, an *in situ* technique[8] was used which provides chromosome spreads on 2 cm^2 coverslips. An average of 50 spreads was counted per preparation. Electrophoresis was conducted on cellulose acetate plates[2].

Fig. 1 Growth rates and longevities of six hybrid cultures. Starting at passage 5 after isolation, 5×10^4 cells were seeded into 75 mm^2 plastic culture flasks and passaged at weekly intervals until there were less of them than input cells after one week's incubation. Estimates of the longevity of these cultures were based on haemocytometer cell counts These counts were converted into averages of population doublings per day as a measure of growth kinetics, and into cumulative population doublings (numbers in parentheses next to the strain identification number) as a measure of total growth potential[4]





Clone # \ Passage #	3	4	5	6	7	8	9	10	11	12
M21-2	100	HP	HP	100	100	HP	100	100		HP
M21-4	41 HP	HP	HP	70		44	25 B	10	10 B	
M21-21	100 HP ●		HP	100	100	HP	100		100 HP	
M22-3	95 HP		100		100 HP	100	100	HP	100	HP
F23-7	100 HP		100 HP		HP					
F24-79	42	HP	20	16 A + B	15	A + B				
F24-85	16	A + B	2	0	0 A + B	4	0 A	A		

Fig. 2 Cytogenetics and electrophoretic profiles of individual hybrid isolates at various passage levels. Numbers express percentages of tetraploid metaphases per culture at a given passage. Densitometric tracings are from electrophoretic separations which were screened in a Beckman R-112 densitometer at 520λ. Otherwise, the G6PD patterns were observed visually and recorded according to the judgment of three independent observers (HP, heteropolymer present; B, G6PD$_B$ phenotype; A, G6PD$_A$ phenotype; A + B, G6PD$_A$ and G6PD$_B$ phenotype, no heterpolymer).

no evidence that other mechanisms[9,10] gave rise to mixoploidy in these colonies.

Of the total of nine isolates containing hybrids (Tables 1 and 2), one was lost due to contamination at the third *in vitro* passage; another consisted of non-replicating cells and, therefore, could not be carried beyond the initial diagnostic step. The seven remaining isolates were subjected to continuous *in vitro* passage and repeatedly analysed. As shown in Fig. 1, the initial growth rates of six of these cultures varied between 0.3 and 0.7 population doublings per day, which is within the usual range for phase II diploid fibroblast cultures in our laboratory. This growth pattern prevailed for an average of five passages, after which the cultures entered phase III of their *in vitro* lifespans (as shown by the irreversible drop of the estimated population doublings per day). Except for obviously random losses of one or two chromosomes and occasional broken and scattered metaphases, only the diploid or tetraploid modes of chromosome counts were observed in all of these cultures. As shown in Fig. 2, three initially 100% tetraploid isolates remained tetraploid throughout their entire *in vitro* lifespans, while also retaining the heteropolymer band.

Table 2 Summary of cytogenetic and electrophoretic classification of clonal isolates from 7 experiments involving a G6PD$_{AB}$ heterozygote strain

Type of fusion: G6PD$_{AB}$ × self (female strain)	Total clones screened/isolated (6,944/136*) G6PD A	G6PD B	G6PD A + B	G6PD Heteropolymer
100% Diploid	6	4	1	
>90% Diploid	1	2	2	
Diploid + tetraploid	16	22	39	2
>90% Tetraploid	6	3	1	
100% Tetraploid	7	14		1

* 9 not tested.
Method as described for Table 1.

Deviations from the symmetrical 1:2:1 pattern seemed in most instances to reflect the evolution of subpopulations in mixoploid isolates. For example, the mixoploid isolates F24-79 and F24-85 (Fig. 2) lost the initial hybrid G6PD during serial culture, which was consistent with their respective cytogenetic evolutions.

These data provide no evidence that hybrids between diploid human fibroblasts regularly eliminate individual chromosomes or chromosome sets, as is the case with hybrids derived from fusions, at least one partner of which is heteroploid. On the contrary, such hybrids seem to proliferate and undergo *in vitro* senescence as chromosomally stable, tetraploid cultures, provided the primary isolates were free of diploid contaminants.

A final point to be made is that the proliferative capacities of the hybrids between human diploid fibroblast strains is not different from other (non-hybrid) tetraploids which were 'aged' as controls, and compares favourably with the usual range of lifespans of moderately vigorous human fibroblast clones of diploid nature[11]. Ultimately, however, the hybrid strains show the finite replicative potentials characteristic of their parents. There is, therefore, no evidence for a complementation of independent genetic defects which might be associated with some somatic mutational theories of clonal senescence[12]. It would, of course, be of great interest to compare the longevities of a series of hybrid clones between parental strains of contrasting longevities, including those from patients with progeroid syndromes[4], although the poor growth of the progeroid parents would pose technical difficulties. The main application of the method, however, should come from the study of genetic complementation in the various human inborn errors of metabolism, which so far has been limited to heterokaryons[13–15], in which there are uncertainties in interpretation. We believe that our non-selective approach can be used without difficulty for the isolation of hybrids between any given combination of euploid fibroblast strains and is able to provide, from a relatively modest group of isolates, quantities of karyotypically stable cells sufficient for a variety of biochemical studies.

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Carcinogenicity of the food additive furylfuramide in foetal and young mice

FURYLFURAMIDE (so-called AF-2), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylic acid amide, had been used widely (probably several tonnes yr^{-1}) as an antimicrobial food additive in such food as fish sausage, mixed meat sausage, boiled fish paste and soybean curd throughout Japan until it was banned in 1974 Tonomura and Sasaki[1] found that furylfuramide induces chromosome aberrations in cultured human lymphocytes and strong mutagenicity was found by Kada[2] and Kondo and Ichikawa-Ryo[3] with *E. coli.* Miyaji[4] had reported, however, that neither tumour nor malformation was induced by furylfuramide in rats and mice, although Morris *et al.*[5] reported that some other nitrofuran derivatives induced tumours in rats. Ames and his associates[6,7] have shown that almost 90% of chemical carcinogens so far tested are mutagenic in their specified tester strains of *Salmonella typhimurium.* Thus, furylfuramide has been an important exception to the presumptive rule that a carcinogen is, in principle, a mutagen. As fish sausage has been favoured so much by young Japanese children, it was necessary to reopen the question of the carcinogenicity of furylfuramide in view of its strong mutagenicity. I report here that furylfuramide induces low but significant yields of tumours in mice when administered at the foetal or young stage.

The experiment with foetuses and newborns (Table 1) was conducted as follows Virgin ICR/Jcl female mice (Japan Central Laboratory for Experimental Animals), 10 weeks old (27–30 g), were placed in a cage with a breeder male at 2200 h, and the next morning the vaginal plug was sought to determine day 1 of gestation. Sixteen pregnant mice (group 1) received three subcutaneous injections of furylfuramide (provided by Drs S. Odashima and I Suzuki, National Institute of Hygienic Science, Tokyo) (dissolved in propylene glycol) at a dose of 50 μg per g body weight, once a day, on days 13, 15 and 17. Newborns of the 21 mother mice (group 2) received a subcutaneous injection of furylfuramide (50 μg per g body weight) within 12 h of birth, and three further injections were given on days 7, 14 and 21 after birth. As a solvent control (group 3), an equivalent volume of propylene glycol was given to pregnant mice or newborns. These offspring were separated from mothers 4 weeks after birth, and maintained on mouse diet CA-1 (CLEA)[9]. They were killed at week 32 after birth, and pathological lesions were examined for tumours as described before[9,10].

Young mice (21 d old and weighing 9–11 g) were divided into five groups (Table 2). The first consisted of 57 male and 51 female ICR/Jcl mice which received three subcutaneous injections of furylfuramide (50 μg per g body weight), once a day, on days 21, 22 and 23 after birth. The second group (25 males and 35 females) received a single subcutaneous injection of furylfuramide at a dose of 100 μg per g body weight on day 21. Solvent controls (group 3) (61 males and 56 females) received an equivalent volume of propylene glycol on the same time schedule. As positive controls (groups 4 and 5), 7,12-dimethylbenz (a) anthracene (DMBA, provided by Drs Odashima and Suzuki) and urethane (Wako) were given to mice on day 21 after birth. Doses of these carcinogens correspond to the maximum tolerance doses in adult ICR/Jcl mice.

When furylfuramide was given to pregnant mice, a significant decrease of live births ($P<0.001$, Table 1) was observed, and only one-third of them lived to the adult stage (8 weeks). The survivors, however, developed significantly higher incidence of lung tumours than controls, whereas tumours were not induced significantly in mice treated during the neonatal and suckling stages (Table 1). Histologically, most lung tumours were papillary adenomas. Three consecutive treatments of young mice with furylfuramide also induced a significantly high incidence of tumours, whereas a single injection did not (Table 2). It is not surprising, however, that Miyaji[4] failed to detect the carcinogenicity of furylfuramide, because the incidence of tumours induced by furylfuramide was very low compared with the commonly used carcinogens DMBA and urethane (Table 2). Some tumours were induced in the forestomach but not in other organs of ddy/SLC mice after feeding on a diet containing furylfuramide for 18 months (Y. Ikeda, personal communication). This may be due to a high rate of decomposition of furylfuramide on the way to other internal organs after direct exposure on the stomach, and/or to the fact that adult mice are in general more resistant to various carcinogens than are foetal and young mice[8,9]. When adult ICR/Jcl mice (10 weeks old and weighing 27–30 g) received three consecutive injections of furylfuramide (50 μg per g each time), lung tumours were not induced significantly (3 of 33 mice (9.1%); 0 12±0.07 tumours per lung, not significant from controls ($P=0.5$)). Furthermore, pretreatment of the young mice with phenobarbital (80 μg per g body weight), which is commonly used for induction of drug catabolising enzymes in the liver and other organs, suppressed the incidence of lung tumours in mice receiving three consecutive injections of furylfuramide (50 μg per g each time) from 29.1% to 4.8% (three of 63 mice treated with both phenobarbital and furylfuramide; 0.06±0 04 tumours per lung, not significant from controls ($P=0$ 8) (T.N , unpublished)) The experiments reported here demon-

Table 1 Effects of furylfuramide on foetal and newborn mice

Experimental groups No.	Age	Dose (µg g^{-1})	No. of pregnant mice	Live births No. (mean)	Lung tumours* Incidence (%)	χ^2 test†	Tumours per lung	*t* test‡	Others
1	Foetus	50×3	16	134 (8.4±0 2)§	7/41 (17 1)	$P<0.02$	0.22±0.09§	$P<0.05$	3 OA
2	Newborn	50×4	21	234(11.2±0.2)	4/43 (9.3)	NS	0.09±0.04	NS	2 OA, 2 TA
3	Control		7	81(11.6±0.5)	2/75 (2.7)		0.03±0.02		

OA, Ovarian atrophy; TA, testicular atrophy; NS, not significant.
*Histologically papillary adenomas. Males and females were not separated, because there was no difference in the incidence of tumours.
†χ^2 test was applied with Yates' correction.
‡*t* test was applied with approximation of Cochran-Cox, if variance ratio was over *F* value at 1%.
§Mean±s.e.

Table 2 Carcinogenicity of furylfuramide on young mice

Experimental groups			No. of	Tumour-bearing mice*		Lung tumours*				
No.	Carcinogens	Dose (μg g^{-1})	mice at start	Incidence (%)	χ^2 test†	Incidence (%)	χ^2 test†	Tumours/lung	*t* test‡	Others
1	Furylfuramide	50 × 3	108	17/55 (30 9)	$P<0\ 001$	16/55 (29 1)	$P<0.001$	0.33 ± 0.07§	$P<0.001$	2 TA, 1 OC
2	Furylfuramide	100	60	1/34 (2.9)	NS	1/34 (2.9)	NS	0.03 ± 0.03	NS	
3	Control		117	2/92 (2 2)		2/92 (2 2)		0.02 ± 0.02		
4	DMBA	20	80	57/62 (91.9)	$P\ll0.001$	50/62 (80.6)	$P\ll0.001$	14.3 ± 2.6	$P\ll0.001$	15 L, 7 F, 2 P 2 SH, 21 OA
5	Urethane	1500	40	32/35 (91.4)	$P\ll0\ 001$	30/35 (85.7)	$P\ll0.001$	16.7 ± 2.5	$P\ll0.001$	1 L, 2 LH, 1 OC

TA, Testicular atrophy; OA, ovarian atrophy; OC, ovarian cystadenoma; L, thymic lymphoma; F, fibrosarcoma (injection site); P, papilloma of skin; SH, splenic haemangioma; LH, liver haemangioma; NS, not significant.

*Mice were killed at week 27 after treatment. Males and females were not separated, because there was no difference in the incidence of tumours.

†χ^2 test was applied with Yates' correction.

‡*t* test was applied with approximation of Cochran-Cox, if variance ratio was more than the *F* value at 1%.

§Mean ± s.e.

strate that even though a chemical has been reported to be non-carcinogenic, if it is later found to be mutagenic there is good reason to re-examine its carcinogenicity in a more sensitive test, such as treatment of foetal or young mice. Mutagenicity in microorganisms is certainly a good indicator for selecting environmental chemicals to be submitted to long term carcinogenicity testing.

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Chromosome aberrations induced in rat lymphocytes by N-nitroso compounds as a possible basis for carcinogen screening

MANY N-nitroso compounds are toxic, carcinogenic, mutagenic and teratogenic, and several are known to give rise to alkylating intermediates in the body[1]. The nitrosamines (for example, dimethylnitrosamine) require activation by enzymes before yielding these intermediates but nitrosamides (for example, N-methylnitrosourea) may react with nucleic acids and proteins without metabolic activation. The nature of the various intermediates between the nitroso compound and the intracellular methylating agent has not been established, however, and the question of the duration of their existence in the body arises. With dimethylnitrosamine, the acute lesion is mainly severe necrosis of the centrilobular zones of the liver, including damage to the hepatic sinusoids and central veins as well as to the centrilobular parenchymal cells[2,3]. It therefore follows that at least one of the intermediates must be of sufficient stability to damage the vascular cells. Since dimethylnitrosamine is active in the host-mediated assay for mutagenesis[4] an active intermediate must also be capable of passing into the peritoneal cavity. N-methylnitrosourea is mutagenic in bacteria[5] and is known to induce chromosomal aberrations in mammalian and plant cells *in vitro*[6,7].

This report describes the induction of aberrations in the lymphocytes of rats treated with dimethylnitrosamine, presumably during their passage through the liver and other organs capable of activating the compound.

As expected, there was a highly significant increase in the number of abnormal cells, that is, those cells containing either chromosomal aberrations or achromatic gaps, from lymphocyte cultures treated *in vitro* with N-methylnitrosourea (0.9 mM), as compared with the abnormal cells from the untreated controls ($\chi^2 = 26.6$, 1 d.f., $P < 0.0005$, abnormal cells containing achromatic gaps have been included in the calculation of significance levels given in the text. Significance levels based on data excluding achromatic gaps are given in Table 1). N-methylnitrosourea seems to induce chromatid type breaks, the vast majority of our aberrations being similar to those already reported for this compound in experiments with *Vicia*[7], that is, chromatid breaks, chromatid exchanges and isolocus breaks. We did find, however, two possible dicentrics, neither of which was accompanied by fragments, in the 200 treated cells. There were no dicentrics in the controls.

This experiment served as a positive control for our next experiment in which rat lymphocyte cultures were treated with dimethylnitrosamine (0.9 mM) *in vitro*. There was no significant difference between the cultures treated *in vitro* with dimethylnitrosamine and the control cultures ($\chi^2 = 1.23$, 1 d.f., $P > 0.2$). In our next experiments we cultured lymphocytes from two animals (A and B) who had been given dimethylnitrosamine 30 mg per kg body weight by intraperitoneal injection, 6 h before being killed. The total numbers of abnormal cells from these animals were in each case significantly higher than the level of their respective controls (animal A: $\chi^2 = 10.75$, 1 d.f., $P < 0.005$; animal B: $\chi^2 = 7.87$, 1 d.f., $P = 0.005$). In these experiments with animals A and B the breaks were again principally of the chromatid type. The significance of these chromatid type aberrations will be discussed later.

Our results clearly indicate that the active intermediate(s) formed metabolically from dimethylnitrosamine have sufficient stability to pass from their sites of formation in the liver and other organs capable of activating the compound and probably to reach the nuclei of the circulating lymphocytes. Since the majority of known chemical carcinogens require metabolic activation[8,9] similar effects on lymphocytes might be expected in animals exposed to them. We are carrying out experiments with other types of carcinogen to investigate this possibility. If this procedure could be developed into a method for carcinogen screening it would have some advantages because the test object is a mammalian cell which is exposed to the metabolic systems for activation of carcinogens by the intact animal rather than the various cellular and subcellular *in vitro* pre-

Table 1 Numbers of rat lymphocytes with chromosome aberrations or achromatic gaps found after *in vitro* treatment with N-methylnitrosourea or dimethylnitrosamine, or *in vivo* treatment with dimethylnitrosamine

	No. of cells scored	Metaphase cells with chromosome aberrations	Metaphase cells with achromatic gaps	Cells scored by two observers (L L. or B.B)	Significance of difference between treated and untreated cultures (excluding cells with achromatic gaps)
N-methylnitrosourea (0.9 mM) *in vitro*	100	18	3	LL	$\chi^2 = 19.56$ 1d.f., $P < 0.0005$
	100	22	2	BB	
Control	100	3	5	LL	
	100	2	0	BB	
Dimethylnitrosamine (0.09 mM) *in vitro*	100	1	0	LL	$\chi^2 = 0.42.1$ d.f., $P < 0.60$
	100	3	1	BB	
Control	100	2	3	LL	
	100	4	1	BB	
Dimethylnitrosamine (30 mg per kg body weight, *in vivo* (animal A))	100	10	2	BB	$\chi^2 = 6.28$ 1 d.f., $P < 0\ 025$
	100	11	5	LL	
Control	100	4	1	BB	
	100	4	1	LL	
Dimethylnitrosamine (30 mg per kg body weight, *in vivo* (animal B))	100	9	2	LL	$\chi^2 = 9.60$ 1 d.f , $P < 0.005$
	100	11	1	BB	
Control	100	3	1	LL	
	100	2	2	BB	

Blood was collected from the abdominal aorta of female Wistar rats, cultured and collected as described[12] previously but with the substitution of foetal bovine serum for human AB serum and the addition of glutamine. In the *in vivo* experiments rats were injected intraperitoneally with dimethylnitrosamine, 30 mg per kg body weight, 6 h before the collection of blood. In the *in vitro* experiments blood was cultured for 24 h before the addition of dimethylnitrosamine, dissolved in 0.9% NaCl, or N-methylnitrosourea, dissolved in 0.01 M citrate buffer, *p*H 6.7, to give final concentrations of 0.9 mM. In both the *in vitro* and the *in vivo* experiments, the cultures were collected at 48 h and slides were coded for blind scoring.

parations currently in use. The sensitivity of the method might be improved by using a sister chromatid exchange method of scoring[10].

We have mentioned that chromatid type breaks were formed after the treatment of rats *in vivo*. Such treatments, of lymphocytes in the G_0 stage of division before DNA synthesis, might be expected to result in chromosome type aberrations appearing in culture, after stimulation. There is evidence, however,—for example, the work of Yoder *et al.*[11] on agricultural workers exposed to pesticides—that exposure *in vivo* can indeed lead to the production of chromatid type aberrations at the first division in culture. It would be interesting to know if the active agent can induce lesions in the G_0 chromosomes which will later be manifested as chromatid-type aberrations, or whether the active agent survives long enough to be carried over, with or in the lymphocytes, to act during the DNA synthesis which follows stimulation in culture. If, however, the active agent should prove to be the methylcarbonium ion then it is difficult to see how the latter hypothesis could be correct.

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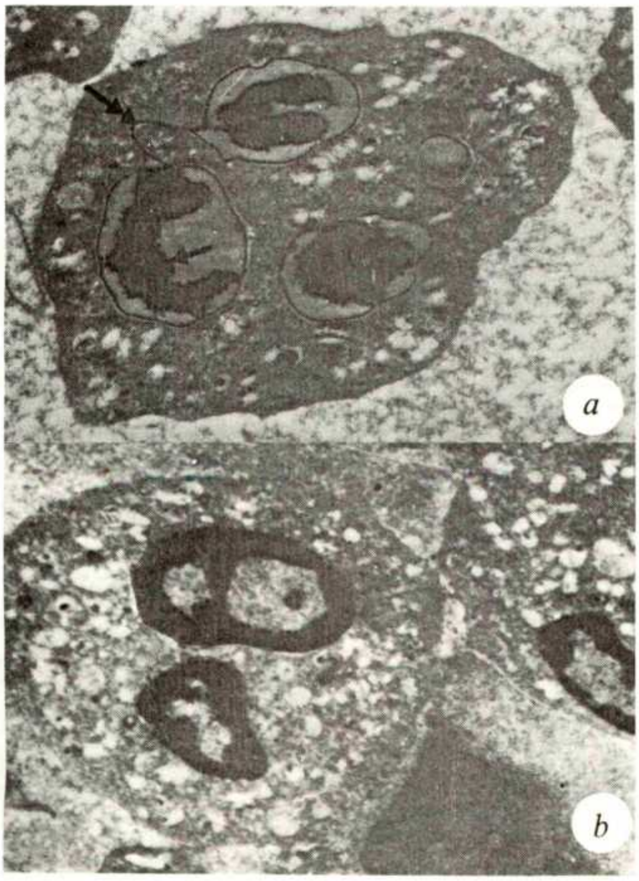
Altered chromatin ultrastructure in neutrophils of schizophrenics

IN spite of reports of altered nuclear morphology of blood cells[1–3] and evidence advocating the participation of genetic factors in schizophrenia[4,5], little attention has been paid to the possibility that altered chromatin is associated with this mental illness Since research in this area may further the understanding of the mechanisms underlying the disease, we compared the chromatin of schizophrenics and normal subjects[6,7], using the neutrophil granulocyte as a model of fully differentiated, non-dividing cells with fixed heterochromatisation. Our approach was based on current knowledge of chromatin[8–10] and focused on the particular roles of the various histone fractions in determining chromatin structure and function[11,12] The results obtained from the initial study[6] showed that schizophrenics differ from controls in their nucleohistone staining pattern. This is characterised mainly by an increased availability of arginine-rich histones[7] to the anionic phosphotungstic acid–haematoxylin (PTAH) staining reagent[13]. We interpreted this as indicating an increased readiness of the nucleohistones in the schizophrenics to undergo conformational changes leading to unmasking of such proteins Considering the role of lysine-rich histone H1 in cross linking the chromatin[11,12,14], we tentatively proposed that in schizophrenics histone H1 tends to dissociate more readily from the chromatin complex

To relate these findings to chromatin fine structure we adapted a histochemical procedure, previously used for smears[7], to electron microscopy (M.R.I. and T.K., manuscript in preparation). We took advantage of the presence of the heavy metal–phosphotungstic acid (PTA) complex, a protein stain for basic residues[15], in the PTAH anionic stain. This could serve as the 'electron stain' and duplicate the histochemical reaction at the ultrastructural level.

We used neutrophil nuclei from 10 schizophrenics and 10 normal controls. All patients were drawn from an experimental group of chronic inpatients at the Corfu State Psychiatric Hospital, who had been drug-free for more than a year and well stabilised in their mental state. They were physically healthy without haematological abnormalities. Controls were physically and mentally healthy volunteers selected from the hospital's resident personnel on the basis that they met the matching criteria on sex and age and shared with the patients more or less the same living conditions and diet. Peripheral blood was obtained on the same day and in the same conditions from patients and controls. EDTA (Versene, 1 drop per 2 ml blood) was used as the anticoagulant. Samples were coded and processed 'blind'. The erythrocytes were permitted to sediment by gravity for 1 h. The leukocyte-rich plasma was removed and kept refrigerated for 24 h. The cells were pelleted and prepared for electron microscopy by glutaraldehyde fixation (see legends to Figs 1 and 2). Double fixation for blood cells with glutaraldehyde and osmium[16], followed by uranyl acetate postfixation[17], was also used. The Epon sections were stained further by uranyl acetate and lead citrate[17].

Fig. 1 Electron micrographs of human neutrophil granulocytes. *a*, Control subject, condensed chromatin in cell appears white (× 7,300). *b*, Schizophrenic subject, condensed chromatin in cell appears black (× 7,300). Both sections are from pellets of cells prepared identically by fixing for 30 min in 2.5% cold glutaraldehyde (buffered with 0.2 M cacodylate), rinsing in cold buffer (twice, 1 h each), treating with 0.25% aqueous $KMnO_4$ at 4 °C for 5 min, rinsing in distilled water, bleaching with 5% oxalic acid for 10 min and washing in tapwater. The pellets were stained in bulk with PTAH (1 g haematoxylin, 20 g PTA 1,000 ml distilled water, 0.177 g $KMnO_4$) for 48 h at room temperature. Dehydration was carried out in a graded series of ethyl alcohol; after two changes of propylene oxide the pellets were embedded in Epon 812. Thin sections were viewed, without further staining, in a Phillips EM 200 electron microscope. In (*a*) two of the lobes are joined by a "nuclear filament" (double-headed arrow) seen as a nuclear envelope-limited sheet. Its strong uptake of PTA indicates a high availability of basic groups in the proteins. The arrow indicates the wavy layer of extended chromatin.



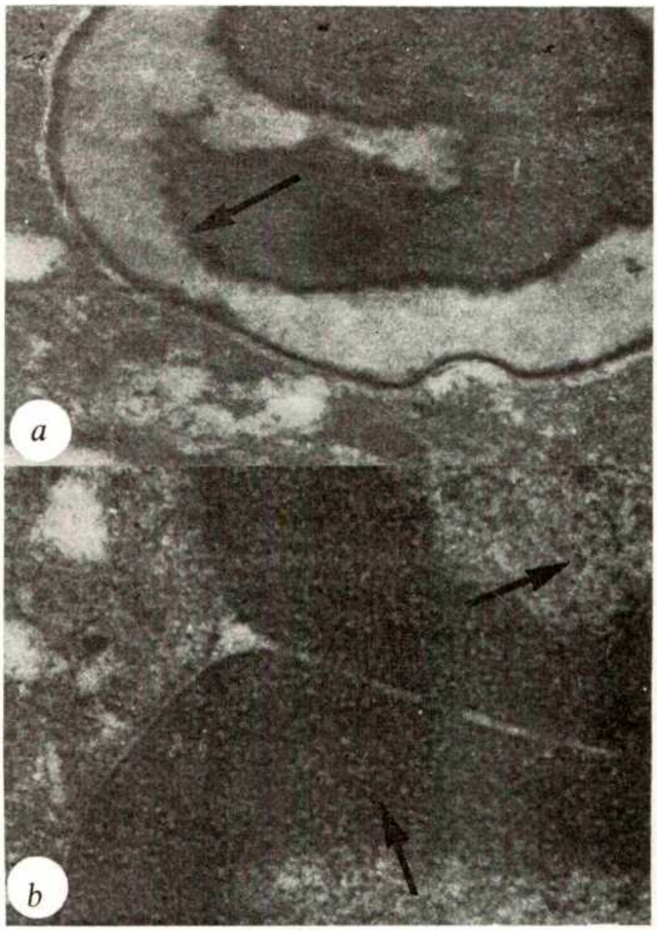


Fig. 2 Electron micrographs of human neutrophil granulocytes. *a*, Control subject, compact chromatin appears white (× 42,900). *b*, Schizophrenic subject, compact chromatin appears black (× 42,900). The cells were prepared as in Fig. 1. The fine structure of the cytoplasm in both (*a*) and (*b*) is identical. In (*a*) the arrow indicates an extended chromatin layer and in (*b*) the arrows indicate individual chromatin fibres.

The PTAH-in-bulk preparatory procedure, but not the osmium–uranium–lead method, revealed differences between patients (Fig. 1*b*) and controls (Fig. 1*a*). In all normal subjects, with the exception of one (Table 1), the area of condensed chromatin of all neutrophils had a clear ground-glass appearance, signifying non-binding of the anionic stain. The dispersed template-active chromatin, however, extending around the periphery of the compact areas (Figs 1*a* and 2*a*, arrows) appeared black, signifying strong PTA binding. Since the basic groups (lysine and arginine residues) are believed to be the binding sites for PTA, these

Table 1 Distribution of subjects according to the pattern of condensed chromatin ultrastructure in their neutrophils

Groups	Type a*	Type b†	Total	χ^2	*P*
Controls ($n = 10$)	9	1	10	16.36	0.001
Schizophrenics ($n = 10$)	0	10	10		

*Condensed chromatin which appears white in Figs 1 and 2.
†Condensed chromatin which appears black in Figs 1 and 2.

results can be understood to depend on the steric configuration of the histone molecules in the nucleoproteins[15,18] and the interaction of the chemicals used (EDTA, $KMnO_4$) with the chromatin[11,19]. A narrow zone around the circumference of the nucleus also showed strong PTA binding and is continuous with the nuclear filament connecting the two lobes of the neutrophil[16] (Fig. 1*a*, double-headed arrow). In contrast to controls, the neutrophils of schizophrenics all had, in the area of condensed chromatin (Table 1), a uniformly high PTA density signifying strong binding of the anionic stain (Fig. 1*b*). The fine structure of the chromatin was sharply outlined as a skein of tortuous fibres 100–300 Å in diameter (Fig. 2*b* arrows). The ultrastructure of the nuclear sap showed a finely granular fairly homogeneous density in the controls, whereas in the schizophrenics it was coarsely fibrous with a light background. Although the preparatory procedure and techniques used were the same for the samples of both groups, the area of compact chromatin in the micrographs appears unstructured white in the controls (Figs 1*a* and 2*a*) and fibrous black in the schizophrenics (Figs 1*b* and 2*b*). This staining pattern indicated that steric hindrance is responsible for the lack of PTA uptake in the nucleoprotein of the controls, probably determined by the state of compaction of the chromatin[18]. Thus the high uptake of PTA in the schizophrenics denotes less compact heterochromatin. This altered state of the chromatin is similar to that observed in the early stages of nuclear maturation[18] when chemical modifications in histones, such as phosphorylation, facilitate their dissociation from the chromatin[20]. Although preparative procedures can cause light and dark appearances of the chromatin in the same cell type[18], the methodological precautions taken in our study rule out this possibility. The differences observed can only be attributed to biological factors.

We conclude that in the neutrophil nuclei of schizophrenics there is a tendency for the chromatin to respond to decondensing stimuli by undergoing conformational changes. If histone H1 can be viewed as the condensing factor of the chromatin[11,12,14] then weak binding due to increased phosphorylation[20] may be considered to be a possible molecular basis of this susceptibility to stimuli leading to the altered state we observed in chromatin. Should further work establish the validity of this postulate, various biochemical findings in schizophrenia (for review see ref. 21) could be explained on the basis of an altered genomic expression and might be unified by a common denominator residing in the chromatin at the interface of gene and environment.

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Different Ly antigen phenotypes of *in vitro* induced helper and suppressor cells

THYMUS-derived (T) cells have a major role in immune systems. They mediate various functions, such as T–B cooperation ('helper cells')[1] and the mixed lymphocyte reaction[2], become killer cells[3], are active in graft *versus* host responses (reviewed in ref. 4) and act as suppressor cells[5]. The identification of the cells responsible for these functions as T cells was facilitated by the use of antisera against the T-cell-specific alloantigen, Thy-1 (ref. 6) (formerly known as θ). It has been reported that Ly alloantigens, present exclusively on T cells[7], identify functionally distinct subpopulations of T cells[8]. For example, T helper cells were lysed by anti-Ly-1, but not by

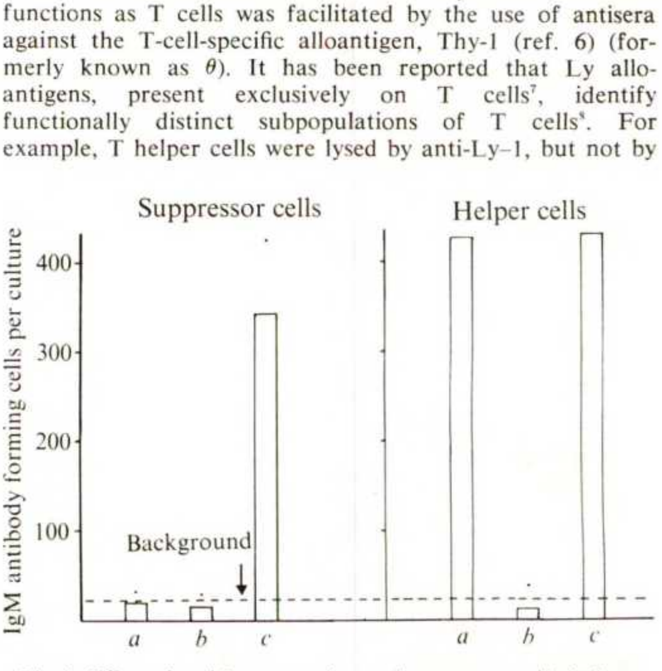


Fig. 1 Effect of anti-Ly sera and complement on specific helper and suppressor cells generated *in vitro* from CBA spleen cells. *a*, Complement; *b*, complement and anti-Ly–1.1; *c*, complement and anti-Ly–2.1. Helper cells were induced as described in ref. 12. Spleen cells were cultured in Marbrook flasks with keyhole limpet haemocyanin (KLH), 0.1 μg ml^{-1} for 4 d with Eagle's MEM containing 5% foetal calf serum. The surviving cells, termed 'helper cells' were assayed for their capacity to cooperate with TNP reactive B cells present in normal CBA spleen in the presence of TNP KLH. Anti-DNP or TNP (di- or trinitrophenyl) antibody-forming cells were assayed using hapten-conjugated anti-SRC Fab′ fragments coupled to sheep red cells (SRC) using the Cunningham plaque assay[14]. Suppressor cells were induced as in ref. 14, culturing (as above) with KLH (100 μg ml^{-1}) for 4 d. Suppressor cells (T cells as judged by the effect of anti-Thy–1 or heterologous anti-T) were assayed by their capacity to inhibit the helper effect of 3×10^5 helper cells. Specificity tests of the suppressor effect are described in ref. 14. Suppressor or helper cells were treated with anti-Ly–1.1 at 1/50 dilution or anti-Ly–2.1 at 1/40, washed and treated with absorbed rabbit complement at 1/5. After treatment cells were counted in Trypan blue. In helper experiments 10^5–3×10^5 viable cells were assayed while in suppressor experiments 10^4–10^5 viable cells were added to 3×10^5 helper cells for assay. For simplicity, results of one cell dose only (3×10^5 helpers, 10^5 suppressors) are shown. Four experiments of this type have been performed. Anti-Ly–2.2 at 1/15 has also been found to inhibit suppressor cells from C57BL (Ly–1.2, 2.2) mice (two experiments, data not shown). Background antigen (TNP KLH) in the absence of helper cells. Dots above bars represents value and 1 s.e.

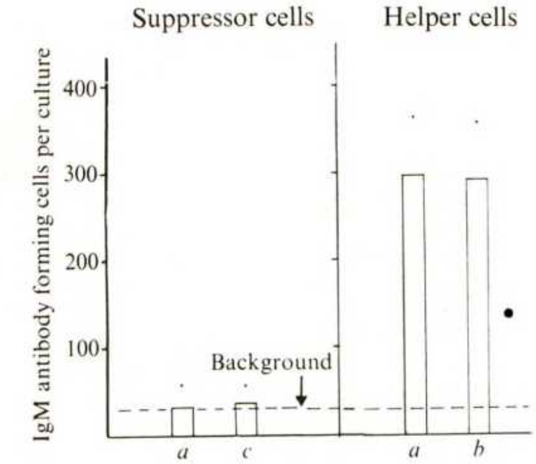
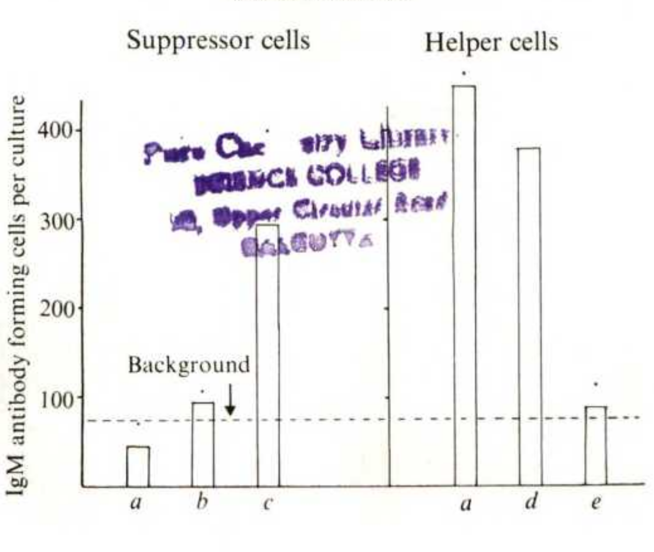


Fig. 2 Functional tests for the specificity of the anti-Ly–1.1 and anti-Ly–2.1 antisera used T helper and suppressor cells raised as described (Fig. 1) from C57BL mice, which like the CBA/Ca were obtained from the ICRF animal unit at Mill Hill, London. Cells were treated with anti-Ly–1.1 or 2.1 and complement as described in the legend to Fig. 1, and 3×10^5 viable helper cells with or without 10^5 suppressor cells were placed in the second culture. No inhibition occurred, verifying functionally the serological specificity of the antisera. *a*, *b* and *c* have the same meaning as in Fig. 1.

anti-Ly–2, so that their phenotype is Ly–1^+2^-, whereas T killer cells were a distinct subpopulation, being Ly–1^-2^+. We report here that specific T suppressor cells induced *in vitro* have a different Ly alloantigen phenotype from T helper cells induced *in vitro* from the same spleen cell pool.

Anti-Ly–1.1, anti-Ly–2.1 and anti-Ly–2.2 antisera were prepared as described before[9]. They were tested for titre and specificity in a two-stage cytotoxic test using rabbit complement, absorbed with mouse spleen cells. If necessary, sera were absorbed with thymocytes of appropriate Ly phenotype to remove autoantibody. Sera were used at dilutions giving plateau levels of cytotoxicity.

Fig. 3 Absorption test of anti-Ly–1.1 and anti-Ly–2.1 with C57BL/6 Ly–1.1 and C57BL/675NS (Ly–1.2, 2.1, 3.1). These mice are congenic to C57BL/6, were obtained from Dr Ian McKenzie, Auston Hospital, Melbourne, Australia and were initially derived by Dr M. Cherry. In this experiment supressor and helper cells were treated with absorbed antisera (see legend to Fig. 1) and 3×10^5 viable helper cells with or without 10^5 suppressor cells placed in the second culture. *a*, complement; *b*, complement and Ly–2.1 absorbed with B6Ly–1.1; *c*, complement and anti-Ly–2.1 absorbed with B10; *d*, complement and anti-Ly–1.1 absorbed with B6Ly–1.1; *e*, complement and Ly–1.1 absorbed with B10.



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The effect of anti-Ly–1.1, anti-Ly–2.1 and complement, or complement alone on *in vitro* induced helper cells of the CBA (Ly phenotype Ly–1.1^+, 2.1^+) mouse strain is shown in Fig. 1. Anti-Ly–1.1 abolished helper activity. Anti-Ly–2.1 had no suppressive effect, confirming the results of Kieselow *et al.*[8]. The effect of the same antisera on specific suppressor T cells, derived from the same pool of spleen cells as the helper cells, is also shown in Fig. 1. Suppressor cells were lysed by anti-Ly–2.1 but not by anti-Ly–1.1. Since the above experiment was not performed with Ly congenic mice, we attempted to verify that the observed differential effect of anti-Ly–1 and anti-Ly–2 antisera were due to their content of anti-Ly–1.1 and anti-Ly–2.1 antibody. First, the anti-Ly–1.1 and anti-Ly–2.1 antisera were tested on populations of helpers and suppressor cells derived from the C57BL strain which bears the opposite Ly alleles—*Ly–1.2* and *Ly–2.2*. They had no effect (Fig. 2). Further evidence of the specificity of the antisera came from absorption studies. Anti-Ly–1.1 antiserum was absorbed with mixed spleen and thymus cells from C57BL/10 (Ly–1.2, 2.2) or C57BL/6 Ly–1.1, and anti-Ly–2.1 was absorbed with C57BL/10 or C57BL 75NS (Ly–1.2, 2.1). The absorbed antisera were tested for their effects on CBA (Ly–1.1, 2.1) helper and suppressor cells. Figure 3 shows that the relevant antibody activity was absorbed as expected, verifying that the antibody activity lysing the suppressor cells was anti-Ly–2.1.

These results demonstrate unequivocally that specific T suppressor cells differ from specific T helper cells, even if both are induced from the same spleen cell pool, in similar *in vitro* conditions, and directed to the same antigen, keyhole limpet haemocyanin. This has three implications. First, it demonstrates that the mechanism of specific T-cell suppression, in this (but not in the other) model, is not due to excess help[10]. Second, it provides a means of selectively manipulating T-cell effects in antibody production (treatment with anti-Ly–2 antiserum has been shown to augment helper activity *in vitro*[11]). Third, our data increase the evidence that differentiated T effector cells have distinctive Ly phenotypes. The relationship of the specific suppressor cells described here to cytotoxic T cells, with the same Ly phenotype, and to nonspecific suppressor cells characterised as Ly–1^+2^+ (ref. 11) remains to be clarified.

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Surface charge modifications associated with proliferation and differentiation in neuroblastoma cultures

THERE have been several reports that malignant cells carry on their surface a higher density of negative fixed charges than normal cells[1-3]. But it has been argued that the differences in observed surface charge reflect differences in cell growth rate rather than malignancy *per se*[4-6]. It has been difficult to resolve this controversy because earlier studies involved comparison between different types of cells, or between cells from the same origin but grown in different culture conditions and at different times after seeding. Using a neuroblastoma cell line[7], we have avoided these shortcomings and made electrophoretic measurements on cells which are homologous from the genetic viewpoint, and which are grown in the same culture tray and collected at the same time, but which exhibit different stages of morphological differentiation.

When neuroblastoma cells are seeded into a Petri dish containing normal culture medium (that is, medium with 10% calf serum), they divide rapidly during the logarithmic phase. Eventually they reach confluence, and contact inhibition causes the culture to enter a stationary phase. Neuroblastoma cells in the stationary phase differentiate and extend processes, and the small, round cells which characterise neuroblastoma tumours *in vivo*[8], as well as the cloned cell line[9] we used, are replaced by large cells, with longer processes, which are more differentiated and resemble sympathetic nerve cells[7-10]. The stationary culture level, however, is not reached in synchronised fashion simultaneously over the entire tray. The cells typically appear in colonies[7,8,10], each with central differentiated cells, with the smaller, round and undifferentiated cells often occupying the periphery (Fig. 1*a*). Thus, in neuroblastoma cultures it is possible to explore differentiated, neurone-like cells, with extensive neurites (Fig. 1*b*), as well as undifferentiated, small, round cells without processes, which are actively dividing (Fig. 1*c*). Both cell types originate from the same seeded cells and thus are genetically homologous; they also bathe in the same culture medium and are collected at the same time, assuring uniformity of the environment. In the experiments described here, cells were collected 2–4 d after the initial seeding. Cells were separated from the Petri dish by directing a fine stream of medium. Cells were then suspended in the medium in which they were cultured previously, and introduced into a cylindrical microscopic electrophoresis apparatus[11,12] (Rank Bros). Measurements were made at 37 °C, and at electric field strength of 2 V cm^{-1}. Care was taken to ensure that all measurements were performed in the stationary layer of the electrophoresis chamber[12]. The *p*H was not controlled during electrophoresis, but the *p*H indicator incorporated in the medium indicated that no gross *p*H shift took place.

Although phase contrast optics have not been used, discrimination between differentiated and undifferentiated cells while the measurements were being taken in the electrophoresis chamber presented no difficulty since undifferentiated, dividing cells as a rule are less than 10 μm in diameter (Fig. 1*c*), whereas differentiated cells generally vary from 20 to 30 μm in diameter, excluding their processes (Fig. 1*b*). To prevent possible error, we restricted measurements to two groups of cells: 8–10 μm in diameter, and more than 25 μm in diameter.

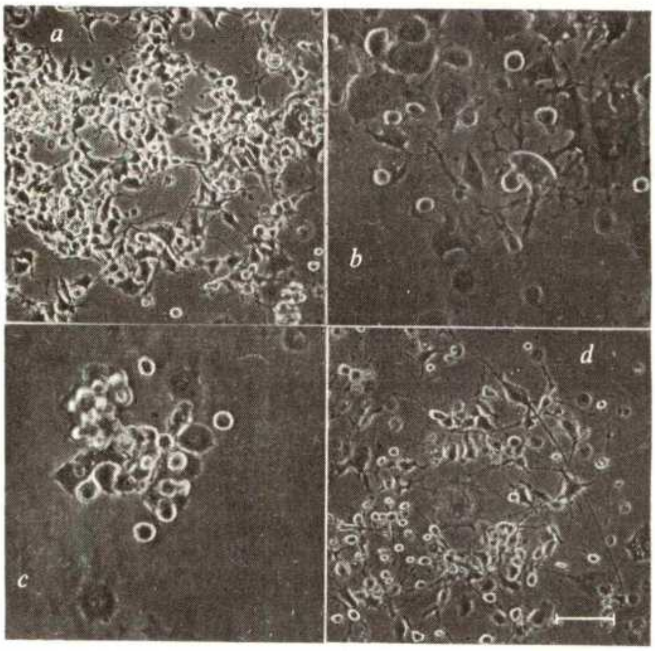

Fig. 1 Neuroblastoma cells in tissue culture. The N-18 clone of C-1300 mouse neuroblastoma cell line was seeded into plastic Petri dishes of 100 mm diameter (Falcon), and grown in Dulbecco's medium containing sodium pyruvate (110 mg l^{-1}). Cultures were incubated throughout at 37 °C, in equilibrium with 5% CO_2 in air. Seeding was adjusted so that at collection the cells would form colonies, but would not reach confluence. Phase contrast photographs of unfixed material, Scale: 100 µm for (*a* and *d*), and 50 µm for (*b* and *c*). Photographs (*a*–*c*) were taken from a culture 2 d after initial seeding, with 10% calf serum in the medium. Photograph (*d*) was taken from a culture 4 d after seeding, grown for the last 2 d with no serum in the medium.

The results are summarised in the first column of Table 1. Clearly the large differentiated cells with processes migrate in an electric field with considerably lower velocity than the small, undifferentiated dividing cells. Both cell types are attracted to the positive electrode, indicating negative zeta potential. Zeta potentials calculated from electrophoretic mobility measurements represent only a lower bound on the total surface charge[13,14], the remainder of the charge being neutralised by adsorption of divalent and polyvalent cations; however, our experiments involve cells grown in the same culture medium in identical conditions, so that adsorption would be likely to be uniform for both differentiated and undifferentiated cells. Moreover, for the study reported here, it is not necessary to calculate the actual zeta potential, since the measurements on both types of cell were made in identical ionic environment and temperature, and since the electrophoretic mobility measured is likely to be directly proportional to the total surface charge[13]. It is possible to conclude directly from Table 1 that the surface charge of undifferentiated cells is approximately 30% more negative than that of differentiated cells from the same culture. A *t*-test on our data indicates that the probability of this occurring by chance is below 0.5%. But, the possibility of systematic error remains. We have found the larger cells to be slower, but for particles with diameters more than 100 times the thickness of the ionic double layer (as have all living cells), the viscosity of the medium has no effect[15]. A more serious difficulty is that while both cell types were collected from the same culture at the same time, the large, differentiated cells have probably been in existence considerably longer than the small, undifferentiated cells which are constantly dividing[7,8], making it necessary to distinguish the effect on the surface charge of cell proliferation from that of the degree of differentiation. This was possible in experiments with cells grown in medium devoid of serum.

Table 1 Electrophoretic mobility of neuroblastoma cells in normal medium and in serum-free medium

Diameter of cell	Normal medium	Serum-free medium
8–10 μm (undifferentiated)	1.27 (±0.05)	1.26 (±0.03)
25–40 μm (differentiated)	0.96 (±0.04)	1.29 (±0.06)

Mobility is expressed in μm s^{-1} cm V^{-1}, and the standard error is given in parentheses next to each measurement.

As reported before, neuroblastoma cells grown in medium not containing serum stop dividing and extend processes[9,10,16]. This was true also in our cultures, and a large percentage of the cells are differentiated, with the cell body more than 25 μm diameter, and with numerous long neurites; nonetheless, there remained some small ($<10\,\mu$m) round cells in these cultures (Fig. 1*d*). Thus it was possible to compare differentiated and undifferentiated cells also in cultures in which cell division was not occurring. The results are given in the second column of Table 1. Clearly the velocity of electrophoretic migration of differentiated and undifferentiated cells in serum-deprived medium is nearly identical.

It might be argued, however, that the differences in surface charge found in serum-containing media reflect differences in adsorption of serum on the surface of dividing and differentiated cells. According to this view, underlying structural differences in membrane structure may persist after withdrawal of the serum, but cannot then be detected electrophoretically.

To examine this possibility, we arrested cell division in the presence of a normal concentration of serum. Cells were grown initially in normal medium containing 10% foetal calf serum. Two days after seeding, cytosine arabinoside (2 μmol l^{-1}) was added to the medium without changing the concentration of the serum. The cells were collected after two further days of incubation in this medium (4 d after seeding). Cultures treated in this way contained both differentiated and undifferentiated cells, but the overall cell density was lower than in cultures grown in serum-containing medium. Cell electrophoresis was performed on cells treated in this way, as well as on control cultures seeded at the same time, but not treated with cytosine arabinoside. No significant difference was found between treated and untreated differentiated cells. The migration velocity of small, undifferentiated cells, however, is reduced by approximately 20% compared with their untreated counterparts. Since cytosine arabinoside is known to arrest cell division, these results support the initial conclusion from the experiments without serum, that the surface charge of the cell membrane is strongly negative shortly after cell division and becomes gradually more positive after the last cell division.

The results reported here suggest that cell proliferation, rather than the level of differentiation, is the significant factor determining surface charge of living cells. Specifically, our data indicate that a high rate of cell division is associated with increased negativity of the cell surface. Considered together with observations on the resting potential of dividing and non-dividing cells (unpublished results of R. E. and Y. Pri-Paz) the results reported here suggest a significant difference in cell membrane structure and function between proliferation and non-proliferating cells from the same cell line, regardless of the level of morphological differentiation.

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Cellular localisation and antigenic species specificity of thymic factors

IT is now generally accepted that T-cell differentiation is influenced by several thymic factors[1,2] and that thymic reticuloepithelial cells are responsible for the production of these factors. Antibodies to a soluble thymic factor (STF) have been reported to inhibit T-cell differentiation in chicken[3] and anti-thymosin globulin has been shown to inhibit the recovery of T cells in ATS-treated mice[4]. Such antibodies therefore can be used to advantage in studies on the modulation of T-cell functions; in this context, a preliminary knowledge of the antigenic species specificity of thymic factors becomes a primary concern.

In this report, we present evidence that bovine thymosin and mouse STF are both found in thymic reticuloepithelial cells of their respective species. Antibodies to these two factors, however, do not cross react.

Anti-STF serum was produced in rabbits according to the technique described previously[5]. Briefly, outbred mice were lethally irradiated and a saline extract was prepared from their lymphocyte-free thymuses (crude STF) which was used for immunisation. The globulin fraction of this antiserum, obtained by ammonium sulphate precipitation[6], was sequentially absorbed with mouse kidney and lung homogenates, bovine kidney homogenates, mouse and bovine thymocyte suspensions and glutaraldehyde-insolubilised whole serum from thymectomised mice. Additional absorptions were performed with kidney homogenates and thymocyte suspensions of rat, hamster and rhesus monkey, on aliquots used for immunofluorescence on these species. Anti-thymosin globulin was produced and absorbed essentially in the same manner as the anti-STF globulin; but using three weekly booster injections instead of only one, after which a positive precipitin reaction was obtained. Thymosin (1mg) (fraction 5) was used for each injection.

Purified STF was prepared by affinity chromatography of the crude STF preparation used for the immunisation, on

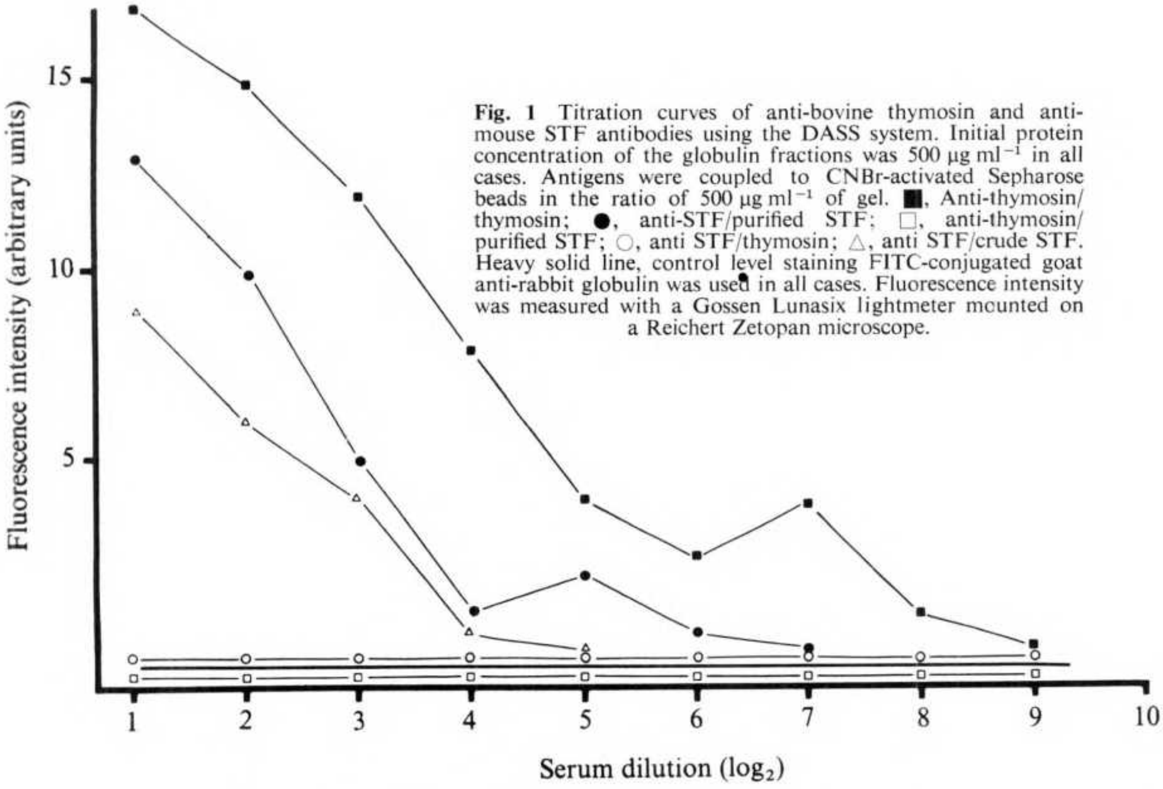


Fig. 1 Titration curves of anti-bovine thymosin and anti-mouse STF antibodies using the DASS system. Initial protein concentration of the globulin fractions was 500 $\mu g\ ml^{-1}$ in all cases. Antigens were coupled to CNBr-activated Sepharose beads in the ratio of 500 $\mu g\ ml^{-1}$ of gel. ■, Anti-thymosin/thymosin; ●, anti-STF/purified STF; □, anti-thymosin/purified STF; ○, anti STF/thymosin; △, anti STF/crude STF. Heavy solid line, control level staining FITC-conjugated goat anti-rabbit globulin was used in all cases. Fluorescence intensity was measured with a Gossen Lunasix lightmeter mounted on a Reichert Zetopan microscope.

columns[2] containing the specifically absorbed anti-STF globulin described above.

The specificity of antibodies to mouse STF and to bovine thymosin was established by the defined antigen substrate spheres (DASS) fluorescence technique[7], with thymosin, crude STF and purified STF as substrates against which antibodies to STF and thymosin were titrated. The titration curves (Fig. 1) show that antibodies to STF reacted with purified STF as well as with crude STF although the reaction with the latter was predictably weaker. No reaction was observed between antibodies to STF and thymosin. On the other hand, antibodies to thymosin reacted strongly and exclusively with thymosin. The same batches of antigen-coupled beads were used for titrations with both antisera and with the control serum.

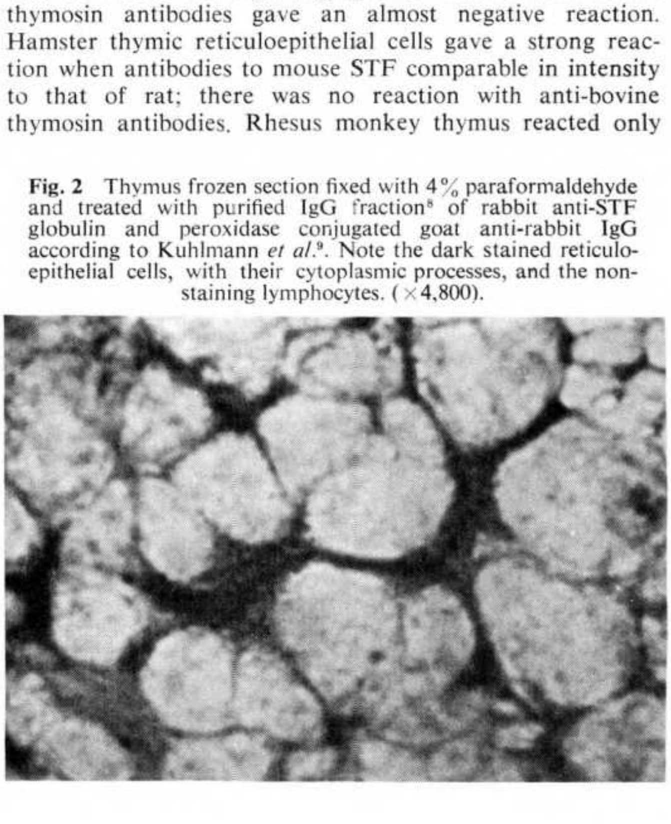
The localisation of STF in thymic reticuloepithelial cells was demonstrated by the immune peroxidase reaction (Fig. 2). With this technique individual reticuloepithelial cells clearly stood out. Numerous positively stained cytoplasmic granules were evident: whether these correspond to vacuoles containing hormone or whether they were a fixation artefact could not be determined by light microscopy.

The antigenic species specificity of thymosin and STF was studied on frozen sections fixed with cold acetone–ethanol by indirect immunofluorescence with FITC-conjugated goat anti-rabbit globulin[5]. Anti-STF globulin on frozen sections of mouse thymus gave a strong fluorescent staining reaction confined exclusively to the reticuloepithelial network (Table 1). The staining pattern was clearly cytoplasmic and uniform: no vacuoles could be distinguished. Occasionally, a negative nucleus was observed and cytoplasmic processes could be seen joining other cells. When anti-thymosin globulin was applied to mouse thymus sections, the reaction was almost negative. Non-immune globulin absorbed in the same manner as the immune globulin gave a totally negative reaction. In this negative control, reticuloepithelial cells could be identified by their slight grey-bluish autofluorescence easily distinguishable from the bright green specific fluorescence. Mouse spleen sections and thymocyte smears when stained with all three sera were negative throughout. Sections of rat thymus gave a reaction pattern with anti-mouse STF globulin similar to that in mouse although slightly less intense. Anti-bovine thymosin antibodies gave an almost negative reaction. Hamster thymic reticuloepithelial cells gave a strong reaction when antibodies to mouse STF comparable in intensity to that of rat; there was no reaction with anti-bovine thymosin antibodies. Rhesus monkey thymus reacted only

Table 1 Antigenic species specificity of thymic factors

Serum	Immunofluorescent staining of thymic reticulo-epithelial cells in				
	Mouse	Rat	Hamster	Monkey	Calf
Anti-mouse STF	++++	+++	+++	(+)	
Anti-thymosin	(+)	(+)			+++
Non-immune					

Fig. 2 Thymus frozen section fixed with 4% paraformaldehyde and treated with purified IgG fraction[8] of rabbit anti-STF globulin and peroxidase conjugated goat anti-rabbit IgG according to Kuhlmann *et al.*[9]. Note the dark stained reticulo-epithelial cells, with their cytoplasmic processes, and the non-staining lymphocytes. (×4,800).



very slightly with anti-mouse STF antibodies and not at all with anti-bovine thymosin antibodies. Calf thymic reticuloepithelial cells on the other hand gave a strong reaction with anti-bovine thymosin but no reaction with anti-mouse STF antibodies.

Our results are in agreement with those of Schulof *et al.*[10] who showed by radioimmunoassay that bovine thymosin cross reacted with sheep, horse and goat but not with mouse, rat and human. It would seem that in all species studied by ourselves and others[11], thymic hormones are synthetised by reticuloepithelial cells and are antigenically similar among related species. The localisation of thymosin obtained by Mandi and Glant[12] is apparently similar to ours but comparisons are difficult since the species used by those authors are not mentioned.

In conclusion, although the biological activity of thymic factors has been shown not to be species specific[1], their antigenic specificity seems to be restricted to closely related species. Care should therefore be exercised in the choice of species combinations in which studies with antibodies to thymic factors are conducted.

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Generalised deficiency of cytochrome b_5 reductase in congenital methaemoglobinaemia with mental retardation

RED cells from patients suffering from congenital recessive methaemoglobinaemia are deficient in a reduced nicotinamide dinucleotide (NADH) diaphorase which has a major role in the process of methaemoglobin reduction[1–3]. This enzyme seems to be identical, or at least related to cytochrome b_5 reductase (EC 1.6.2.2) for the following reasons: (1) a red-cell-type NADH diaphorase has been found in several different non-erythroid cells from normal human subjects, indicating that it is a widely distributed enzyme[4]; (2) cytochrome b_5 reductase has been found in the red cells, and cytochrome b_5 seems to have a direct role in methaemoglobin reduction[5,6], (3) in patients with NADH diaphorase deficiency a concomitant deficiency of cytochrome b_5 reductase has been observed[7,8]. In a small but significant proportion of the cases of congenital enzymopenic methaemoglobinaemia, the methaemoglobinaemic syndrome is associated with a progressive neurological disorder with mental retardation causing death at a young age[3,8,9]. The relationship between methaemoglobinaemia and nerve system involvement has so far been poorly understood, until the finding that the leukocytes of patients with this type of disease also displayed the NADH diaphorase deficiency, whereas nearly normal enzyme levels were found in the leukocytes from methaemoglobinaemic patients without neurological symptoms[8,10] It was therefore postulated that methaemoglobinaemia with neurological involvement might result from a generalised enzyme defect[8,10].

Here we demonstrate that this is indeed the case by finding a lack of both NADH diaphorase and cytochrome b_5 reductase in several tissues: red cells, leukocytes, muscle, liver and fibroblasts from a patient suffering from methaemoglobinaemia with mental retardation.

The clinical pattern displayed by the propositus, a 1-yr-old female child, was very similar to that previously reported in other cases of methaemoglobinaemia with mental retardation[3,9] There was no detectable methaemoglobin reductase activity[11] in the red cells. Traces of enzyme retaining a normal mobility were, however, detected on electrophoresis of a concentrated haemolysate followed by specific staining for NADH diaphorase[12]. The level of enzyme activity in the red cells of both parents was in the heterozygous range: father: 2.5 U per g haemoglobin (Hb); mother: 2.4 U per g Hb (normal controls: 4 5±0.8 U per g Hb; 1 U corresponds to 1 μmol ferrocyanide-methaemoglobin complex reduced per min (ref 11)).

To verify the hypothesis of a generalised enzyme defect in this disease, several tissues were investigated. The leukocytes were prepared by PVP sedimentation followed by osmotic shock. Muscle and liver specimens were obtained by surgical biopsy from the propositus, and from a patient of the same age suffering from another condition. Skin fibroblasts were cultured in Eagle's MEM containing 10% calf serum. Cells were collected on day 3 after three passages. All tissue fragments and the pellets of leukocytes and fibroblasts were frozen in dry ice immediately after collection, and stored at −70 °C. The homogenates were prepared with a Potter–Elvejhem apparatus in a 10 mM potassium phosphate buffer, *p*H 7.5, and sodium deoxycholate (0.5% final) was subsequently added. In the case of leukocytes and fibroblasts, the pellet was pretreated by repeated freezing and thawing before homogenisation. The

Table 1 NADH diaphorase (methaemoglobin reductase) and cytochrome b_5 reductase activities in several tissues of the propositus

Tissues	Methaemoglobin reductase		Cytochrome b_5 reductase	
	Propositus	Control	Propositus	Control
Red cells (Hb-free extract)	0	144	< 0 05	2 0
Leukocytes	2.3	90	< 0.05	2.9
Muscle	6 0	64	< 0.05	2.4
Liver	5.0	94	< 0.05	3.6

Leukocyte, muscle and liver extracts were prepared as described in the text. Hb-free red cell extracts were prepared by DEAE–Sephadex treatment[13]. Methaemoglobin reductase activity was assayed according to Hegesh *et al*[11], and expressed as μmol ferrocyanide–methaemoglobin complex reduced per min per mg protein. Cytochrome b_5 was prepared from liver microsomes according to Omura and Takesue[14]. Cytochrome b_5 reductase activity was assayed according to Mihara and Sato[15]. The reduction of 10 μM cytochrome b_5 was followed at 556 nm in the presence of 180 μM NADH, and the activity expressed as the first-order rate constant (min^{-1}) per mg protein. Several other enzymes chosen as internal standards were also measured: 6-phosphogluconate dehydrogenase in the Hb-free red-cell extracts; aldolase, hexokinase, phosphoglucomutase, glucose-6-phosphatase and ten lysosomal enzymes in the liver extracts. They were all in the normal range (data not shown).

assays of methaemoglobin reductase activity[11] and of cytochrome b_5 reductase activity[15] were carried out on the 30,000*g* supernatant.

From the results summarised in Table 1, it is clear that both enzyme activities are lacking in the propositus, not only in red-cell extracts, but also in leukocytes, muscle and liver. The enzyme defect could also be found in the patient's cultured fibroblasts (Table 2), suggesting that antenatal diagnosis might be possible. To check this possibility, we investigated the cytochrome b_5 reductase activity in control cultured amniotic cells. Our results indicate that these cells possess an easily measurable activity, comparable with that found in normal fibroblasts derived from skin (Table 2). The diagnosis of homozygous deficiency should therefore be feasible in cultured amniotic cells.

Table 2 Cytochrome b_5 reductase activity in cultured fibroblasts

Cells	Cytochrome b_5 reductase activity
Skin fibroblasts from the propositus	< 0.05
Skin fibroblasts from control subjects	4 10
Control cultured amniotic cells	8

Enzyme activity measured and expressed as described in Table 1. Mode of extraction as described in text.

These results bring the first experimental evidence for a generalised enzyme defect in congenital methaemoglobinaemia with neurological involvement. In addition they provide further indication that NADH diaphorase (so-called methaemoglobin reductase) and cytochrome b_5 reductase are probably identical entities, since both activities were found to be defective in various tissues. This is consistent with previous observations made in normal tissues[4], and in red cells from normal[5,6] and methaemoglobinaemic subjects[7,8]. Another possibility is that the two enzymes share a common subunit.

The precise role of cytochrome b_5 reductase is still not established. In the red cells, where it is present in a soluble form, this enzyme would act as a methaemoglobin reductase[5,6]. Elsewhere it is mainly located in microsomes, and has been particularly studied in liver[16] and in brain[17]. A soluble form has also been described in rabbit liver cytosol[18]. The relationship between the microsomal and the soluble enzyme is not known. Our results now indicate, however, that they are controlled by the same gene since both forms are defective in the propositus: the soluble form in the red cells, the particulate form in deoxycholate-treated liver, muscle and leukocyte extracts. This is in agreement with the immunological relationship that we have found between the cytosolic and the microsomal cytochrome b_5 reductase (A.L. and J.-C.K., unpublished). A generalised deficiency of cytochrome b_5 reductase is expected to produce a systemic impairment of the metabolic processes linked to cytochrome b_5, such as fatty acid desaturation[19]. Since the major impact of the disease seems to be on the central nervous system, it would be very important to analyse the fatty acid composition of brain lipids in such patients.

Thus the molecular basis of congenital methaemoglobinaemia seems to be a defect of the soluble cytochrome b_5 reductase in the form without brain involvement, expressing as a red-cell disease, and a combined defect of both the cytosolic and the microsomal cytochrome b_5 reductase in the form with mental retardation, manifesting as a generalised disease.

The explanation for the two types of cytochrome b_5 reductase deficiency is still speculative. If the enzyme is coded by a single gene, several hypotheses can be made: (1) the abnormal gene product is unstable, but produced at a normal rate, and only mature red cells, which cannot synthesise proteins, are affected; if the mutation causes drastic instability or underproduction of the enzyme, the defect is generalised; (2) some mutations only impair the formation of the soluble enzyme from a microsomal precursor, and the enzyme defect is only expressed in the red cells, whereas other mutations produce a defect of both the microsomal and the soluble forms of enzyme, thus causing a generalised disease. If two genes are implicated, one can postulate that methaemoglobin reductase and cytochrome b_5 reductase share a common subunit which is defective in the generalised disease.

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Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis

IN androgen-resistant male mice of genotype *Tfm*/Y spermatogenesis is arrested at first meiotic division. This is likely to be due, at least in part, to the need for testosterone if spermatogenesis is to proceed beyond prophase of first meiosis[1]. It would be somewhat surprising if the response to androgen involved any gene action in the developing sperm cell itself, since the locus of the gene for response to androgen, *Tfm*, is on the X chromosome[2]. The X–Y chromosome pair is believed to become genetically inactive during meiotic prophase[3], and at stages beyond meiosis I only 50% of spermatocytes and spermatids possess an X chromosome.

To test this point we made male mice chimaeric for androgen-resistant (*Tfm*/Y) and normal (+/Y) genotype by the technique of embryo aggregation[4,5], and tested their fertility to determine whether spermatozoa were formed from the *Tfm*/Y component and were able to take part in normal fertilisation. Of 57 visibly chimaeric animals produced, 2 fertile males proved to be of the correct *Tfm*/Y ⟷ +/Y genotype and both produced numerous offspring from their *Tfm*/Y component, indicating that the requirement for testosterone in spermatogenesis does not involve the cell-autonomous action of the *Tfm* locus in the germ cells.

In two series of experiments embryos from matings of *Tfm*+/+*Ta*×*Ta*/Y were fused with homozygous pink-eye (*pp*) or albino (*cc*) embryos, chosen so that the coat colour variegation in the resulting chimaeras would give some visual indication of the proportions of the two components, at least in the pigment cells. The albino stock also carried the chromosome marker Rb 1Ald. The gene tabby (*Ta*), which is closely linked to *Tfm*, was used as a marker in the *Tfm* stock.

The *Tfm* component of any animal could be of four possible types: *Tfm*+/+*Ta*, +*Ta*/+*Ta*, *Tfm*/Y or +/Y (if the rare crossovers between *Tfm* and *Ta* are neglected), whereas the other component could be XX or XY. The genotype of any particular animal was established by a combination of visual inspection and breeding tests. The presence of the *Ta* gene might be detected in the coat of animals with *Tfm*+/+*Ta*, +*Ta*/+*Ta* or *Ta*/Y components, and some evidence of resistance to androgen might be seen in male animals carrying either *Tfm*+/+*Ta* or *Tfm*/Y components. Drews *et al.*[6] have described the intersexuality of chromosomally XX animals, heterozygous for the *Tfm* gene, and sex-reversed to male by the gene *Sxr*, and, since chimaeric animals commonly show phenotypes somewhat similar to those of heterozygotes for X-linked genes[7], we expected to observe intersexuality of some kind in our chimaeras.

Table 1 Animals produced from two series of embryo aggregations

	Series 1 (*pp*)			Series 2 (*cc*)		
	♀	♂	*Tfm*/Y	♀	♂	*Tfm*/Y
Non-chimaeric	9	12	8	3	1	—
	♀	♂	Intersex	♀	♂	Intersex
Chimaeric						
pp or *cc* only*	3	—	2	10	6	6
Ta only	5	3	—	—	—	—
Ta and *pp* or *cc*	4	7	—	5	4	2

Matings of *Tfm*+/+*Ta*♀×*Ta*/Y♂ and of PT♀ (genetically *aa bb* $c^{ch}c^{ch}$ *dd pp ss sese*) by *pp*♂ (or in the second series of *cc* Rb 1Ald Rb 1Ald ♀ by *cc* Rb 1Ald Rb 1Ald ♂) were detected by inspection for vaginal plugs Two days later the eggs (at the four to eight-cell stage) were flushed from the oviducts into medium by the usual techniques[8] and the zonae pellucidae were removed by treatment with pronase. One egg of each type—*Tfm* and *pp* or *cc*—was introduced into a drop of medium under oil, and the two eggs were pushed together. The eggs were then cultured overnight at 37 °C The following day the eggs were washed in medium and those apparently viable embryos in which good fusion had occurred were transferred to the uterus of a pseudopregnant recipient female (of C3H/HeH×101/H hybrid type) which had shown a vaginal plug by a sterile male 2 d previously. Usually, only one uterine horn of each female was injected, with four or five embryos. *Visible variegation in coat.

We did indeed find 10 animals with some degree of intersexuality of the genitalia (Table 1), ranging from a mainly female phenotype with masculinised clitoris, through various degrees of ambiguity, to a mainly male phenotype with small teats present. If as expected these animals had a *Tfm*/Y or *Tfm*+/+*Ta* component there should be little evidence of the *Ta* gene in the coat, and indeed only two of the 10 showed slight expression of *Ta*, whereas 14 out of 20 true males showed *Ta*. The six intersexes judged to be sufficiently masculinised to be potentially able to mate as males were placed with *pp* or *cc* females and these females were inspected for vaginal plugs daily. On this criterion three males mated repeatedly and of these two, both from the *cc* series, proved to be fertile. One was only barely fertile, and has so far produced only 16 offspring in five litters, all albino, that is from his *cc* component. Chromosome tests on cultures of ear skin showed him to have XX and Rb 1Ald XY cell lines and he was therefore presumed to be *Tfm*+/+*Ta* ⟷ *cc* XY, although no *Ta* stripes were visible in his coat. The other was fully fertile and produced young from both his components, that is 21 pigmented and 45 albino. The pigmented offspring were all non-*Ta*, and all of four females genetically tested proved to be *Tfm*/+. This is as expected if the chimaeric parent's pigmented component was *Tfm*/Y, and since he produced both sexes of offspring from both cell lines this male was proved to be of the genotype *Tfm*/Y ⟷ *cc* XY. In addition, all the phenotypically normal males with no signs of intersexuality were bred from and all proved fertile. Thirteen produced young from their pigmented component and in all cases except one all the daughters were *Ta*/+ (that is the pigmented component was *Ta*/Y). The one remaining animal, again from the *cc* series, with no intersexuality and no sign of *Ta* in his coat, produced 26 pigmented young, all non-*Ta*, and 27 albino. All five pigmented daughters which were genetically tested proved to be *Tfm*/+, that is this chimaeric male also was proved to be *Tfm*/Y ⟷ *cc* XY.

In the first of the two fertile *Tfm*/Y ⟷ *cc* XY chimaeras the *cc* component apparently predominated, since his coat was at least 95% *cc*, he was fully masculinised externally (that is of the non-*Tfm* type) except for a pair of small abdominal teats, and his offspring (as already mentioned) were about 67% *cc* and 33% *Tfm*. In the other animal, however, as much as 50% of the coat and of the offspring were of the *Tfm* type, although as previously mentioned he was fully masculinised externally.

Thus, these two males both provide evidence that male germ cells genetically resistant to testosterone through the presence of the *Tfm* gene are capable of normal maturation and fertilisation—the requirement for testosterone in spermatogenesis does not involve the cell-autonomous action of the normal allele of the *Tfm* locus in male germ cells.

Various possible mechanisms for the normal maturation of *Tfm*/Y sperm in a chimaeric testis with genetically normal cells present can be envisaged. The first is that the *Tfm* locus is not involved in the response of the seminiferous epithelium to androgen, and that the arrest of spermatogenesis in *Tfm*/Y non-chimaeric animals is due to cryptorchidism or other secondary factors. This seems unlikely as the *Tfm* locus has been shown to be involved in almost all other responses to testosterone[9,10].

Another possibility is that the *Tfm* locus is involved but acts not in the germ cell, but in another cell type, for example the Sertoli cell or the tubular epithelial cells. The role of the Sertoli cells in the maintenance of spermatogenesis by testosterone is well established. One product of the Sertoli cells is an androgen binding protein (ABP) secreted into the lumen of the seminiferous tubules[11]. Clearly in a chimaera such a free protein would be available to germ cells of both components. Although ABP is androgen-dependent[12] it is not, however, clear that it is involved in spermatogenesis or that it is determined by the *Tfm* locus, which is known to control a cytosol androgen-receptor[13–15]. Such a receptor is present in the seminiferous tubules, but Hansson *et al.*[16] could not establish whether it was produced by the Sertoli cells or the germ cells.

Thus, there is the third possibility that in the *Tfm*/Y ⟷ +Y chimaeras the relevant protein could be passed from one germ-cell type to the other. This seems unlikely in view of the high fertility and relatively high proportion of *Tfm*-type young produced by our two males. Although it is known that intercellular bridges occur normally both among spermatogonia[17] and in spermatids, these are presumably between cells descended from a single stem cell and therefore genetically from the same component of a chimaera. There is no reason to suppose that cooperation between developing sperm from different stem cells could occur to a sufficient degree to rescue 50% of the fertilising sperm, as in our second animal. On the other hand, derivation of the necessary protein from Sertoli cells or other tubule cells seems much more plausible. Since these cells are derived embryogenically from a different cell population, *Tfm*/Y germ cells may easily have been in close contact with +/Y Sertoli cells and moreover the proportion of the +/Y component in the Sertoli cells of the two fertile chimaeras may well have been higher than in the germ cells. Further information may be obtainable later from histological sections of the testes, but at present the mice are needed for breeding homozygous androgen-resistant daughters.

Thus, these data support but do not conclusively prove that the response to androgen of the seminiferous epithelium is entirely mediated by testicular somatic cells. Similarly, testosterone is required for normal maturation of spermatozoa in the epididymis[18,19], and our data again indicate that action of the *Tfm* locus in the spermatozoa themselves is not involved. If, as seems likely from Drews and Alonso-Lozano's[20] study of the epididymis in *Tfm*/+ *Sxr*/+, gene action at this locus in the epididymal cells is necessary, then clearly in a chimaera a sufficient proportion of +/Y cells can ensure maturation of spermatozoa from both components.

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Electrical properties of 'dark green' and 'yellow' neurosecretory cells in the snail *Lymnaea stagnalis* L.

A NUMBER of electrophysiological investigations of neurosecretory neurones have been carried out in gastropod molluscs[1–6] but in every case except one[5] the neurosecretory status and function of the cells was uncertain. In the pulmonate snail *Lymnaea stagnalis* L , substantial morphological and histochemical evidence obtained by light and electron microscopy exists for regarding the 'dark green' (DGCs) and 'yellow' (YCs) neurones as neurosecretory[6–8] The distinction between DGCs and YCs was based on their colour reaction to a combined Alcian blue–Alcian yellow staining technique used on sections of fixed brain material viewed under the light microscope[7], but differences in elementary neurosecretory granule diameter and axon terminal location have also been found[7,8]. Good evidence also exists for a function of DGCs and YCs in ionic or osmotic regulation in *Lymnaea*; the rate of release of both DGC and YC neuroendocrine material increased when snails were kept in deionised water and decreased in hypertonic saline[8–10]. We show here that it is possible to identify DGCs and YCs in electrophysiological experiments and characterise them on the basis of their location, appearance and electrical properties in the live brain

Large specimens of commercially supplied *Lymnaea stagnalis* (2.9–6.2 g) were kept in Brighton tapwater and fed on lettuce. Electrophysiological experiments were carried out on isolated brains (from 83 animals) immersed in blood just previously collected from the intact snail. Some dissection of loose connective tissue surrounding the brain was often necessary to see the superficially situated neuronal cell bodies, and the inner sheath surrounding the brain was treated for short periods with Protease (Sigma, Type V) to aid intracellular penetration of neuronal cell bodies with microelectrodes. Electrical signals were recorded intracellularly from neuronal cell bodies and fed into a Bioelectric Instruments amplifier (Farmingdale, USA) which allows bridge balance of electrode resistance and current injection through the recording electrode while recording resulting changes in membrane potential. Electrodes were filled with 2 M K_2SO_4 or 6% Procion Yellow M-4RS. Recorded potentials were displayed on a storage oscilloscope and permanently recorded on film or pen recorder.

To identify the neurones from which recordings had been made as YCs or DGCs, their colour reaction to Alcian blue–Alcian yellow (AB–AY) treatment in histological preparations was investigated. Candidate neurones were marked with Procion Yellow by iontophoresis from the recording electrode in the conventional manner[11]; only one cell per ganglion was injected. The brain was fixed in Stieve's fluid, embedded in wax, serially sectioned, and the sections mounted on slides without coverslipping The marked cells were found under ultraviolet light in the light microscope, photographed and the cell body locations carefully noted. The sections were then stained by the AB–AY method[7] and the staining reaction of the cells recorded.

Dark green cells were found on the ventral surface of the two pleural ganglia where, from morphological studies, they are known to occur[7]. They were 30–50 μm in soma diameter *in vivo*, and their orange colour was often similar to the surrounding non-neurosecretory neurones[12] In some preparations they were orange with a slight white tint in reflected white light, but never the white or blue-white of other *Lymnaea* neurosecretory cells[13] Twenty-nine DGC were marked with Procion dye and subsequently confirmed by AB–AY staining.

DGCs always showed low frequency steady firing (Fig 1*a*) at mean rates within the range 10–60 action potentials per min. Spike shape was conventional, with duration 12–20 ms (at 50% spike height) with spikes showing a pronounced after-hyperpolarisation (Fig 1*b*). Spike shape and firing pattern were quite different from YCs (Fig. 2). Depolarising postsynaptic potentials were seen in DGCs when the cells were hyperpolarised (Fig. 1*c–e*). Their amplitude was increased by hyperpolarisation and as they did not reverse polarity at threshold for impulse initiation,

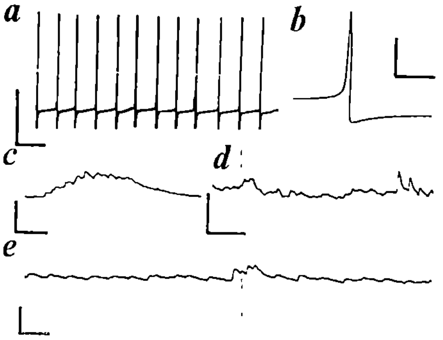

Fig. 1 Electrical properties of 'dark green' neurosecretory cells from *Lymnaea*. *a*, Example of low frequency spike activity recorded in all DGCs; *b*, DGC action potential on fast time base to show impulse shape with its prominent after-hyperpolarisation; *c–e*, e.p s p s recorded in DGC whose spike activity was suppressed by application of steady hyperpolarising current. *c*, Compound e p.s.p of long duration, about 10 s *d*, Continuous high frequency e p.s.ps. input to DGCs causes irregular baseline fluctuations where individual e.p s p s are difficult to distinguish except for two unitary large e.p.s p s (largest one, 10 mV) towards the end of the trace. *e*, Continuous e.p s.p s of low amplitude (1–2 mV) and around 500 ms duration typical of those recorded in most DGCs Occasional larger e p.s p s are imposed on this basic input. Scale bars: *a*, 2 s, 40 mV; *b*, 100 ms, 40 mV; *c*, 2 s, 10 mV; *d*, 1 s, 10 mV; *e*, 1 s, 5 mV.

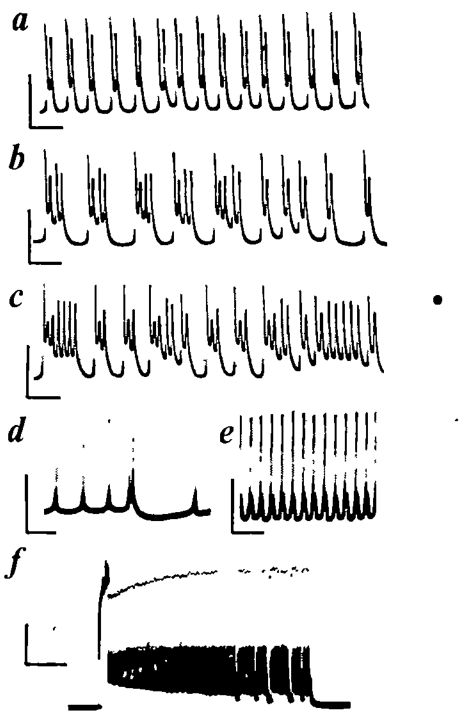

Fig. 2 Intracellular recordings from 'yellow' neurosecretory cells from *Lymnaea*. *a–c*, Spontaneous firing of a YC from the ventromedial surface of the right pleural ganglion. The cell was gradually depolarising so that in *a* doublet spikes were predominant, whereas at higher levels of depolarisation grouped firing of mixed doublets and triplets (*b*) were seen which changed to longer duration burst at the highest level of depolarisation shown (*c*) YCs at just suprathreshold polarisation for spike initiation often generate single spikes with occasional doublets (*d*). Note the larger hyperpolarisation following the doublet spike compared with the single. At very high levels of depolarisation, YCs show a high frequency continuous single spike pattern (*e*). *f*, An example of a phenomenon seen in a few YCs: the cell was silent but a single depolarising square pulse of current which had to be large enough to generate spikes produced a long self-sustaining burst of spikes lasting about 15 s. Towards the end of the burst doublet spikes were formed. Scale bars: *a–c*, 1 s, 30 mV; *d*, 1 s, 40 mV; *e*, 1 s, 30 mV; *f*, 2 s, 20 mV.

they are chemically mediated excitatory postsynaptic potentials (e.p s p.s) One type of e.p.s p. was continuous in occurrence and of low amplitude (1–3 mV) (Fig 1*e*), whereas a second type of e p s.p occurred sporadically but was of larger amplitude (4–10 mV) (Fig. 1*d*). Occasionally, large compound e.p.s.p s were recorded, which presumably resulted from high frequency bursts in presynaptic cells (Fig 1*c*).

Yellow cells occurred in the ganglionic locations noted by Wendelaar Bonga[7] but also commonly on the medial surface of the pleural ganglia They were 50–70 μm in soma diameter *in vivo*, and, unlike DGCs, were usually clear white in colour or sometimes orange-white when viewed in reflected white light. Their white axons could often be seen traversing the ganglion surface to the connectives and sheath tissue surrounding the brain. Twenty-one marked YCs were identified by AB–AY staining but 40 others were identified on the basis of position, colour and electrical properties, once these were known.

The distinctive feature of YCs is their tendency to generate double spikes (Fig. 2) These either occurred spontaneously or could be generated by setting the cells at the appropriate level of polarisation The double spikes were best seen when the cell membrane potential was just suprathreshold for impulse initiation (Fig 2*a*) At higher levels of applied depolarisation, or spontaneously, bursts of spikes were observed which consists of mixed doublets, triplets and single spikes (Fig. 2*b* and *c*) At high levels of depolarisation, the cells fired in a continuous single spike pattern (Fig. 2*e*). Some YCs when firing at low frequency (less than one spike every few seconds) also generated single spikes with occasional doublets (Fig. 2*d*). An interesting variation in YCs bursting tendency was found in four cells. They were silent, but a single suprathreshold depolarising square pulse of at least 0.5 s duration induced a self-sustaining burst of impulses which lasted up to 45 s (Fig. 2*f*). The burst consisted mostly of single spikes, but a few double spikes formed part of most bursts in a particular cell. No synaptic input was seen which was responsible for the spike activity seen in many YCs, which thus seems to be endogenous in origin.

The results show that the DGCs and YCs are distinct in appearance and electrical properties and we can now recognise these cells *in vivo* without the need for dye marking and subsequent staining.

Roubos[9] has shown that the secretory responses of DGCs to deionised water and saline solutions can occur in isolated ganglia when these are transplanted into experimental animals Receptors mediating these responses must therefore lie within the brain, and these may be the source of excitatory input to the DGCs. Previous electron microscopic evidence had not revealed the presence of synapse on DGCs[9].

The tendency of YCs to fire bursts of spikes of variable height is very distinctive and has not been seen in other molluscan neurosecretory neurones which burst, such as R15 of *Aplysia*[4] or cell 11 of *Otala*[2].

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Marked prolongation of post-tetanic potentiation at a transition temperature and its adaptation

A UNITARY, monosynaptic excitatory postsynaptic potential (e.p s p.) can be recorded from cell R15 of the abdominal ganglion of *Aplysia californica* on appropriate stimulation of the right visceropleural connective. The amplitude of this e.p s p. undergoes a sequence of changes with trains of one or two stimuli per second. These changes include synaptic depression, frequency facilitation and post-tetanic potentiation. As described in detail elsewhere[1,2], all these phenomena are apparently due to alterations in the amount of neurotransmitter released rather than to postsynaptic mechanisms. The frequency facilitation, a relatively transient phenomenon, is believed to be limited by the rate of neurotransmitter mobilisation into the pool available for release[2]. In contrast, the post-tetanic potentiation, a much more sustained phenomenon, is believed to be due to a change in the fraction of the available pool of transmitter released per stimulus[2].

To evaluate further the relationship of frequency facilitation to post-tetanic potentiation (PTP) we studied the effects of changing the temperature of the preparation. We found that the effects of temperature provided another means for distinguishing the mechanism underlying these phenomena. A more striking finding was the discontinuous relationship between temperature and the duration of PTP. This provided clues to the mechanism of PTP.

The abdominal ganglion of *A. californica* was pinned in a dish, and perfused with modified seawater as described previously[1,2]. The temperature of the preparation was maintained within ± 0.25 °C with a thermoelectric module (Melcor) regulated by a thermistor placed close to the ganglion. Cell R15 was hyperpolarised to eliminate endogenous bursting; stimulation and intracellular recording were as described before[1,2]. Preparations were stimulated at 2 pulses s^{-1} for 100 stimuli. When the train stopped, test pulses were given every 20 s to obtain an approximation of the maximal amplitude of the e.p.s.p. during the PTP period Thereafter, test pulses were given at 10-min intervals to obtain an estimate of the duration of PTP.

We found that the duration of PTP, which was slightly prolonged by reducing the temperature from the usual 15 °C to 12 °C, was strikingly prolonged when the temperature was reduced to 10 °C (Figs 1 and 2*a*) or 8 °C. The relationship of the duration of PTP to temperature is, therefore, discontinuous in that a transition temperature is observed. In four control experiments in which no trains of stimuli were given we found that the amplitude of an e.p.s.p. elicited every 10 min remained constant when tested for 4 h each at 10 °C and 8 °C. Therefore, the enlarged e.p.s.p. amplitude observed after a train at 10 °C and 8 °C is due to prolongation of PTP rather than to temperature effect on an e.p.s.p. elicited every 10 min, as is done when testing the duration of PTP.

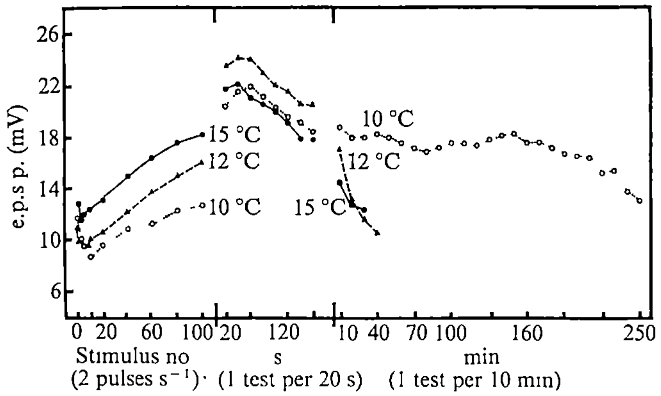

Fig. 1 Amplitude of the unitary and monosynaptic e.p.s.p. obtained in cell R15 on minimal stimulation of the right visceropleural connective. The following stimulus pattern was given sequentially at 15 °C, 12 °C and 10 °C: 100 stimuli at a frequency of 2 s^{-1} followed by eight test pulses at 20-s intervals and then by test pulses every 10 min until the size of the e.p.s.p. had returned to the initial level. The temperature was then lowered and the preparation was allowed to equilibrate for 30 min without stimulation before the stimulus sequence was repeated. Neither the amplitude of the first e.p.s p. of a train nor the maximum size of the PTP were significantly affected by a change in the temperature. The frequency facilitation during the trains showed a fairly linear temperature dependence, while the duration of the PTP was abruptly prolonged between 12 °C and 10 °C.

In contrast to the findings with PTP duration, the amplitude of frequency facilitation (measured as the ratio of the hundredth e.p.s.p. of the train to the first) showed a relatively smooth dependence on temperature (Fig. 1 and Table 1), the amplitude of the first e.p.s.p. of a train and the maximal amplitude of the e.p.s.p. measured during the PTP were not significantly affected (Table 1).

Since poikilothermic animals such as *Aplysia* show adaptation to changes in ambient temperature[3], we determined the effects of maintaining the animals at 11 °C rather than the usual 20 °C for 2 d before removal of the ganglion. We found that the duration of PTP no longer showed a transition at 10 °C or 8 °C. In several animals, however, there appeared to be a transition when the temperature was reduced to 6 °C (Fig. 2*b*). We also found that when the isolated ganglion was maintained for 4 h at 10 °C before stimulation, the duration of PTP did not show a transition at several low temperatures (Fig. 2*c*).

Temperature dependence of various neurophysiological phenomena, such as the duration of PTP at neuromuscular junctions[4,5], has been reported. The effects of temperature on frequency facilitation that we observed are typical of the temperature dependence of many enzymatic reactions—that is, a linear dependence on temperature over a wide range[3]. The nonlinear relationship between temperature and duration of PTP is, in contrast, more reminiscent of reactions in systems where activity depends on the state of fluidity of lipids. For example, membrane transport systems that contain lipid and protein components show a striking change in a level of function at transition temperatures[6–8]. Although transition temperatures in protein function in the absence of lipids are also possible, most known phenomena of the type observed here are due to phase changes in lipids at critical temperatures. The rapid adaptation observed here could be related to known responses to temperature change that could affect protein structure, lipid fluidity or both. Changes in lipid composition of membranes in bacteria, with altered membrane function, have been observed in less than an hour[6,9].

These considerations raise the possibility that PTP is due to a change in the state of organisation of a membrane component of the presynaptic nerve terminal; and that the rate of decay of this new state of membrane organisation is strikingly retarded at a temperature at which there is a reduction in fluidity of the lipids associated with this state

Table 1 Effect of temperature on an isolated e.p.s p. maximal PTP and frequency facilitation

Temperature (°C)	8	10	12	15	20
Isolated e.p s.p.*	0.94±0.10	0.93±0.06	1.00	1.02±0.06	1.01±0.10
Maximal PTP†	0.93±0.12	1.00±0.05	1.00	0.98±0.05	0.96±0.08
Frequency facilitation‡	0.72±0.06	0.88±0.05	1.00	1.04±0 06	1.12±0.11
No. of preparations	3	10	10	6	3

All values and ratios are expressed as relative to the measurement at 12 °C, which is set at 1.00.

*The amplitude of the first e p s.p. of a train. None of these groups differed significantly.

†Defined as the ratio of the amplitude of the maximum measured e.p.s.p during the PTP period to that of the first e.p.s.p. of the train. Results are shown relative to the measurement at 12 °C. None of these groups differed significantly.

‡Defined as the ratio of the amplitude of the last e p s.p. of the train to the first e.p.s.p. of the train. Results are shown relative to the measurements at 12 °C. The groups at 8 °C and 10 °C differed significantly ($P < 0.05$) from the 12 °C group. The groups at 15 °C and 20 °C differed significantly ($P < 0.025$) from the 10 °C group.

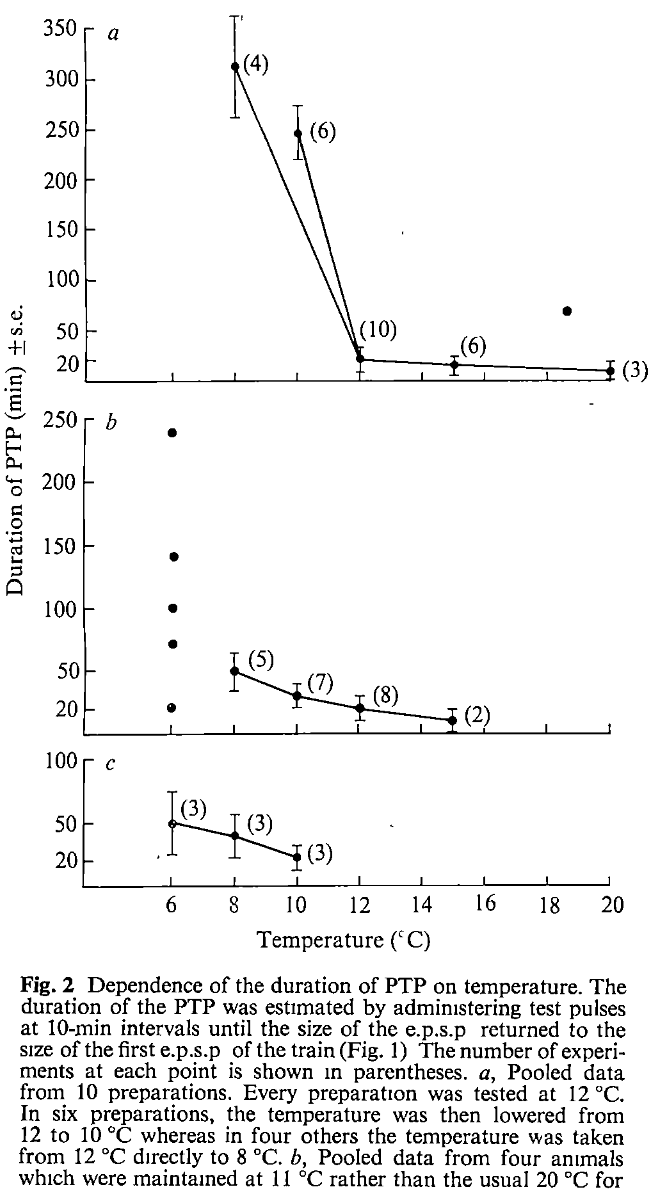

Fig. 2 Dependence of the duration of PTP on temperature. The duration of the PTP was estimated by administering test pulses at 10-min intervals until the size of the e.p.s.p returned to the size of the first e.p.s.p of the train (Fig. 1) The number of experiments at each point is shown in parentheses. *a*, Pooled data from 10 preparations. Every preparation was tested at 12 °C. In six preparations, the temperature was then lowered from 12 to 10 °C whereas in four others the temperature was taken from 12 °C directly to 8 °C. *b*, Pooled data from four animals which were maintained at 11 °C rather than the usual 20 °C for 2 d before the experiment The duration of the PTP was only slightly prolonged when the temperature was lowered from 12 °C to 10 °C or 8 °C but at 6 °C some preparations showed an abrupt prolongation of the PTP. None of the preparations treated in this fashion showed a temperature transition between 12 and 10 °C. *c*, Three isolated ganglia were maintained at 10 °C for 4 h before stimulation. In these conditions the duration of the PTP measured at 10 °C was short compared with control animals (*a*) and no abrupt prolongation occurred when the temperature was lowered to 6 °C.

of membrane organisation. Consistent with this possibility is the finding[10] that ethanol (4%) and butanol (0.1%), agents that increase membrane fluidity[11,12], increase the rate of decay of PTP at 15 °C about fivefold Some presynaptic membrane functions that might be critical to these findings include (1) regulation of calcium levels in the nerve terminal by calcium uptake functions of mitochondrial membranes[13], and (2) regulation of fusion of synaptic vesicles with release sites on the plasma membrane through changes in the lipid–protein order of the presynaptic membrane[14]. PTP might be due to a change in the state of order of one of these membranes as a result of repetitive stimulation; the decay of PTP might then be retarded by treatments that decrease membrane fluidity (low temperature) and accelerated by treatments that increase membrane fluidity (alcohols).

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Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates

It is well documented that barbiturates dramatically prolong presynaptic inhibition[1–4]. Their effect on postsynaptic inhibition is less clear, although the available evidence suggests that a similar enhancement may occur at some sites[5–7]. Since the inhibitory postsynaptic potentials (i p s p s) in hippocampal neurones are unusually large and the neurones are readily penetrable by microelectrodes[8], we have studied the effect of barbiturates on these i.p.s.p.s in detail. We have found that barbiturates hyperpolarise hippocampal neurones and markedly prolong the i.p.s.p. by a direct action on inhibitory synapses, anaesthetic doses increasing the duration fivefold The hyperpolarising action of barbiturates and their effect on i.p s.p.s would both contribute to the neural depressant action of these anaesthetic agents.

Cats were anaesthetised either with 80% nitrous oxide and 0.3% Halothane or with an intraperitoneal injection of pentobarbitone (35 mg kg^{-1}) The animals were paralysed with Flaxedil and artificially ventilated, the end tidal CO_2 being maintained at 3.5%. A bilateral pneumothorax and cisternal drainage were carried out Femoral blood pressure was continuously monitored and the femoral vein was cannulated for the administration of barbiturates After cutting a window in the skull, the hippocampus was exposed by removing the overlying cortex with suction. Intracellular recording was limited to CA_1 neurones. Stimulation with a local surface electrode produced a large i.p.s p in these neurones[8] Immobilisation of the hippocampus was secured by the following procedures A thin layer of 4% agar in Ringer's solution was placed on the surface of the hippocampus. A conical shaped pressure foot was placed on the agar and the surrounding area was entirely filled with low melting point wax which formed a rigid connection with the bony edges of the skull. The microelectrodes were filled with KCl 2.4 M, potassium methylsulphate 2 M, or potassium citrate 2 M No difference in the effect of barbiturates on the i p.s.p. was noted with these different salts, although the i.p.s.p. was usually depolarising with the KCl-filled electrodes. The electrode resistance varied from 20–50 MΩ. The resting membrane potential and i.p.s p. size were continuously monitored on a pen recorder and film records were obtained at appropriate intervals.

In the first series of experiments the i.p.s.p.s recorded from a number of neurones during 80% nitrous oxide anaesthesia were compared with the i.p.s.p.s recorded after the animal had been given 30 mg kg^{-1} pentobarbitone intravenously and the nitrous oxide had been turned off. The activity of neurones in the latter group was recorded within 2 h after the pentobarbitone injection, only neurones having membrane potentials greater than −35 mV being used. With nitrous oxide anaesthesia the mean half-decay time of the i p s.p.s was 26.8 ms±10.2 s.d. (n=33), whereas with pentobarbitone anaesthesia it was 128.3 ms±28.4 s.d. (n=24). The degree of prolongation was independent of the resting membrane potential. These results show that in the presence of pentobarbitone the i p.s p. is prolonged greatly.

To examine these effects in more detail, barbiturate was injected while recording from neurones. The results are based on 29 neurones. With eight of these neurones the i p.s.p s were recorded during nitrous oxide anaesthesia and subsequently after barbiturate was injected. In the remainder, the control recording was in a pentobarbitone-anaesthetised animal and the effect of a supplemental injection was examined. The results from these two groups of neurones were similar although, as expected, less dramatic in the latter group In 16 neurones the recording conditions were so stable that it was possible to examine the effect of barbiturate on the resting membrane potential. In five neurones barbiturate injection effected no change in membrane potential. In the other 11 neurones the injection resulted in a slowly developing hyperpolarisation, averaging 5.6 mV±2.1 s.d. which was independent of the initial resting membrane potential. The dose of pentobarbitone injected in these experiments varied from 5 to 33 mg kg^{-1}.

The intravenous injection of pentobarbitone produced a dose-dependent prolongation of the i.p.s.p. which was independent of any change in the membrane potential. Figure 1*a* shows the typical action of pentobarbitone on the membrane potential and i.p.s.p. of a hippocampal neurone. The membrane potential increased from 50 to 60 mV. The amplitude of the i.p.s p. increased twofold while the half-decay time of the i.p.s.p. gradually increased from 38 to 160 ms. Although a fall in membrane potential occurred at 37 min, presumably due to partial dislodgement of the electrode, there was only a slight transient change in the duration of the i.p.s.p. In Fig. 1*b*, 15 mg kg^{-1} pentobarbitone increased the half-decay time of the i.p.s.p. from 27 to 60 ms without any change in membrane potential. The minimum effective dose of pentobarbitone for this effect was 5 mg kg^{-1}. The relative increase in the half-decay time for approximately 10 mg kg^{-1} had a mean value of 2.15 times ±0 7 s d (n=16). Thiopental (Fig. 1*c*) and thiamylal (not illustrated) also prolonged the i.p s.p A similar lengthening was seen on i.p.s.p.s which had been reversed by diffusion of chloride ions from the microelectrode (Fig. 1*d* and *e*). The results on reversed i.p.s.p s were obtained after the i.p s.p. had stabilised; therefore, the prolongation cannot be attributed to chloride ions diffusing to more distant inhibitory synapses. In Fig. 1*e*, the stimulus strength was just above threshold and numerous reversed spontaneous unitary i.p.s.p s can be seen, particularly in the background responses of *f*. Pentobarbitone (10 mg kg^{-1}) prolonged both the evoked and spontaneous unitary i.p.s.p.s in e_2 and f_2.

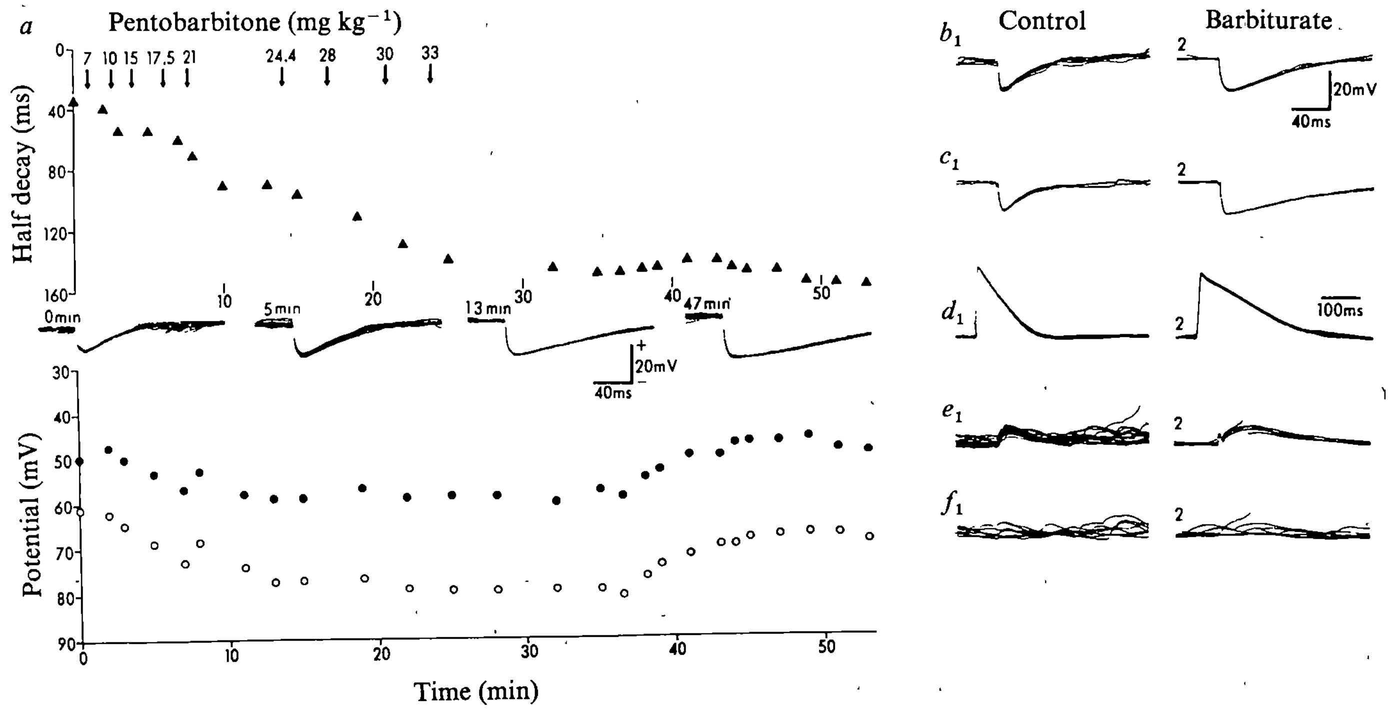

Fig. 1 Prolongation of i.p.s.p.s by barbiturates. *a*, Top graph half decay of the i.p.s.p. (▲); bottom graph membrane potential (●) and peak of the i.p.s p. (0) The time scale applies to both graphs. Sample film records, taken at the indicated times are shown between the graphs. The cumulative dose (mg kg^{-1}) of pentobarbitone after each injection is shown at top of figure. In *b*, 15 mg kg^{-1} pentobarbitone was injected into a nitrous oxide-anaesthetised cat. The membrane potential remained stable at −37 mV and b_2 was obtained 5 min after b_1. In *c*, 17 mg kg^{-1} thiopental was injected into a nitrous oxide-anaesthetised cat. The membrane potential (−50 mV) remained stable over the 12-min period between c_1 and c_2. The i.p.s.p. in *d* was depolarising due to diffusion of chloride ions from the microelectrode with the consequent high level of intracellular chloride. Pentobarbitone 10 mg kg^{-1} was injected into a pentobarbitone-anaesthetised cat. The membrane potential (−55 mV) was constant during the time (8 min) between d_1 and d_2. The i.p.s.p. in *e* was also reversed by diffusion of chloride ions from the microelectrode. The background recordings, *f*, from this same neurone show numerous reversed unitary i.p s.p.s Pentobarbitone 10 mg kg^{-1} was injected into a pentobarbitone-anaesthetised cat and membrane potential was increased from −43 to −45 mV. In e_2 an action potential was elicited from one of the i.p.s p s after pentobarbitone. The time between the two responses was 4 min. The time and voltage calibration in *b* applies to all records except for the time calibration in *d*.

The hyperpolarising action of barbiturates on many hippocampal neurones might arise from a number of factors including withdrawal of background excitation[9], blockage of intracellular oxidative metabolism[10] and activation of γ-aminobutyric acid (GABA) receptors[11].

In a previous study on field potentials in the olfactory bulb[7], it was proposed that barbiturates prolonged post-synaptic inhibition. The present intracellular study on hippocampal neurones provides direct evidence that this is indeed the case. The effects of barbiturates on post- and pre-synaptic inhibition are qualitatively and quantitatively similar. Interestingly, GABA is thought to be the transmitter involved both in pre-[2,12,13] and in postsynaptic inhibition at supraspinal sites[14,15], including the hippocampus[16,17]. This barbiturate effect could result from prolonged release of inhibitory transmitter, a direct action on the GABA conductance mechanism or delayed removal from postsynaptic receptor sites. The observation that pentobarbitone can prolong the action of directly applied GABA[11,18] would suggest that prolonged release is not the sole basis for the barbiturate effect. The finding that pentobarbitone is a competitive blocker of GABA uptake[19] suggests that the prolongation may result from delayed removal from postsynaptic receptor sites.

In the past, emphasis has been placed on the depressant action of barbiturates on excitatory synaptic transmission[20–24] If the dramatic prolongation of hippocampal i.p.s p.s by anaesthetic doses of barbiturate (approximately four to five times) applies to other inhibitory synapses of the cerebral cortex, a general depression of the excitability of the cerebral hemispheres would be expected. The prolongation of the unitary postsynaptic inhibitions would lead to more effective summation of repetitive inhibitory actions and so account for, at least in part, the action of barbiturates in producing unconsciousness.

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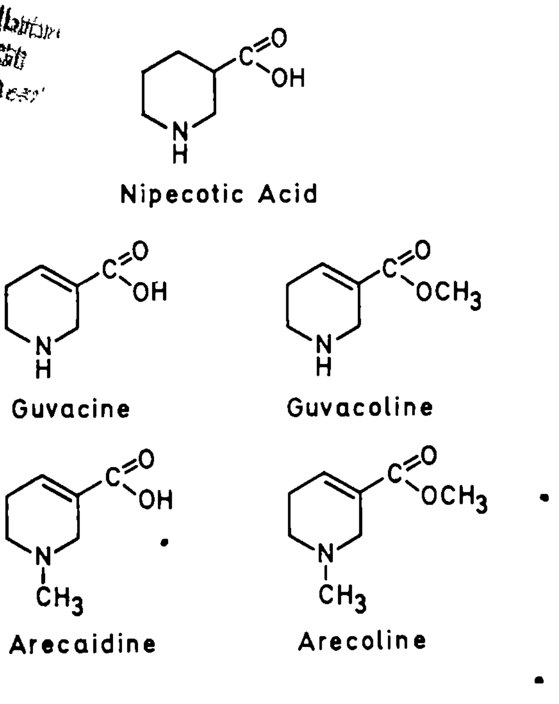
Betel nut constituents as inhibitors of γ-aminobutyric acid uptake

THE pharmacological properties of betel nut, the dried seed of *Areca catechu* which is consumed in immense quantities in the East as a masticatory, are usually interpreted in terms of the cholinomimetic effects of the major alkaloid component, arecoline. Betel nut contains lesser amounts of the related alkaloid guvacoline and the amino acids arecaidine and guvacine. According to popular tradition, a mixture of crushed betel nut and lime is enveloped in leaves of *Piper betle* before chewing. This treatment with lime has been shown to hydrolyse almost quantitatively arecoline to arecaidine[1], a well tolerated substance which lacks the typical parasympathomimetic effects of arecoline, including tremor and salivation[1,2]. Studies on the effects of arecaidine on the behaviour of mice indicate that this substance participates in producing some of the psychic changes ascribed to the betel mixture[1,2]. These betel constituents are tetrahydronicotinic acid derivatives (Fig. 1) and we have shown that nipecotic acid, 'hexahydronicotinic acid', is a potent inhibitor of the uptake of the central inhibitory transmitter γ-aminobutyric acid (GABA) in rat brain slices[3]. Nipecotic acid potentiates the depressant action of microelectrophoretically administered GABA on the firing of feline spinal neurones[4]. As the uptake of GABA is likely to be concerned with the inactivation of this transmitter, interference with GABA uptake could result in behavioural changes. The structural similarities between nipecotic acid and the betel nut constituents together with the above mentioned findings prompted us to examine the influence of these compounds on GABA uptake. We have found that arecaidine and guvacine are competitive inhibitors of GABA uptake in rat brain slices and we propose that some of the psychic effects of betel nut consumption may be the result of inhibition of GABA uptake.

The 'high affinity' uptake of GABA was studied in slices of rat cerebral cortex using the procedure developed by Iversen and Neal[5]. The high affinity uptake of L-glutamate, glycine, and L-proline was studied in a similar manner[6–8]. Arecaidine was prepared from commercially

Fig. 1 Structures of nipecotic acid, guvacine, guvacoline, arecaidine and arecoline.



available arecoline by acidic hydrolysis[9]. Guvacine was prepared as described by McElvain and Stork[10], and guvacoline was prepared from this by esterification according to Freudenberg[11]. The four compounds were analytically pure, and their infrared and proton magnetic resonance spectra were consistent with the structures in Fig. 1.

Arecoline and guvacoline (0.5 mM) had no effect on the uptake of GABA or L-glutamate. Arecaidine and guvacine (0.5 mM) inhibited the uptake of GABA by 75±3 and 98±1% (mean±s.e.), respectively, but did not inhibit the uptake of L-glutamate, glycine or L-proline. Probit analysis of the results of experiments with appropriate concentrations of arecaidine and guvacine indicated that 50% inhibtion of GABA uptake was obtained with 122±12 μM arecaidine and 8±1 μM guvacine. On this basis, guvacine was as potent as nipecotic acid[3], which inhibited GABA uptake by 50% at 9±1 μM.

All of these experiments were carried out with preincubation of the inhibitor with the brain slices for 15 min before addition of radioactive GABA. As has been observed with nipecotic acid[12] and 2,4-diaminobutyric acid[13], less apparent inhibition of GABA uptake was obtained if arecaidine or guvacine were added at the same time as GABA. This may be interpreted on the basis of these inhibitors being substrates for the GABA uptake system and entering intracellular compartments during the preincubation period. Kinetic studies showed that arecaidine and guvacine act as competitive inhibitors of GABA uptake when added at the same time as GABA. The apparent slope inhibition constants (K_{is}) were 141±38 μM for arecaidine and 14±4 μM for guvacine. On this basis, guvacine has a similar affinity for the GABA carrier to nipecotic acid[3] which has a K_{is} of 11±3 μM.

These experiments are consistent with arecaidine and guvacine acting in rat brain slices as substrate-competitive inhibitors of GABA uptake, binding to the GABA carrier and penetrating the tissue. Such an action *in vivo* could contribute to the observed effects of arecaidine administered to mice, which includes reduction in spontaneous activity, exploration and motility[2]. Antagonism of the inactivation of GABA might lead to prolongation of GABA-induced synaptic inhibition, such as that proposed for pentobarbitone, which at 5 mM inhibits (58%) the uptake and potentiates (46%) the release of GABA in rat brain slices[14]. Arecaidine, injected subcutaneously into mice in doses of 50–100 mg kg^{-1} (0.3–0.6 mM if evenly distributed in body fluids), has been shown to prolong barbiturate narcosis and to inhibit spontaneous activity[2]. We have shown that guvacine, injected intraperitoneally into mice in doses of 50–100 mg kg^{-1} provoked a pronounced but rather short lived reduction of spontaneous activity, the duration of action being 15–30 min.

Guvacine is more potent than arecaidine with respect to inhibtion of GABA uptake *in vitro*. The above mentioned results may stimulate further studies on the pharmacology of guvacine and arecaidine, and preparation of structural analogues of these amino acids as potential psychoactive agents.

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Persistence of tetracycline resistance in pig *E. coli*

THE practice of feeding tetracyclines to pigs to promote growth was prohibited in Britain in March 1971, after a period of some 17 yr during which this practice had been common. It was prohibited because it had encouraged the emergence of antibiotic-resistant strains of bacteria in pigs. A comparison of the results of examining pigs brought to Chelmsford Market in 1956, 1970 and July 1972 indicated that during 16 months prohibition the incidence of pigs harbouring tetracycline-resistant *Escherichia coli* in their faeces had not decreased but that the amount of these organisms in the pig population might have done so[1]. To obtain additional information, similar surveys were done in July 1973, July 1974 and July 1975; the result of all six surveys are collated in this paper.

On each occasion, individual faecal specimens from 100 pigs in different pens were collected; this ensured that a high proportion of the pigs had come from different farms. The methods of assessing the antibiotic sensitivity of *E. coli* in the specimens, of recording the results and of identifying transmissible resistance in *E. coli* strains have been described previously[1]. So has the method of identifying strains harbouring non-transmissible antibiotic resistance determinants that can be mobilised by transfer factors F, I or A2 (refs 2 and 3).

The results of the sensitivity tests, summarised in Table 1, suggest that in the four years since prohibition the amount of tetracycline-resistant *E. coli* in the pig population might have decreased slightly but that the incidence of pigs excreting these organisms (100% in 1975) had not. The failure of prohibition to achieve a greater impact may be because during the long period in which the *E. coli* in the pig population had been exposed to tetracycline, resistant strains had emerged which were now able to compete on equal terms with sensitive strains. The decrease since 1970 in the proportion of tetracycline-resistant strains whose resistance was transmissible (Table 2) suggests that, in a comparatively tetracycline-free environment, possession of a mechanism for transmitting tetracycline resistance is, in general, disadvantageous. The tetracycline-resistance determinants in 9 of 15 non-transmitting tetracycline-resistant *E. coli* strains isolated from different pigs in the 1973 survey could be mobilised by transfer factors F, I or A2. The continued veterinary use of tetracycline could also have contributed to the failure The veterinary use of streptomycin and sulphonamides was undoubtedly responsible for a substantial amount of the streptomycin and sulphonamide resistance arising during the study period because these two antibiotics have not been used as feed additives. The use of either antibiotic could have contributed to this situation because of 144 streptomycin-resistant strains isolated from different pigs in the 1972–75 surveys, 127 were also sulphonamide resistant, probably because streptomycin and sulphonamide-resistance determinants are commonly linked on the same plasmid. The use of these antibiotics could also have made some contribution to the survival of tetracycline-resistant *E. coli* because 63 of the 144 strains previously mentioned were tetracycline resistant and 28 of 146 strains selected because they were tetracycline resistant were streptomycin resistant. The use of streptomycin was probably

Table 1 Antibiotic sensitivity of *E. coli* in faecal specimens of pigs

Antibiotic	Year when specimens were collected	% of specimens in which the *E coli* were: All resistant	Resistant and sensitive	Nearly all sensitive	All sensitive
Oxytetracycline	1956	18	13	22	47
	1970	64	22	7	7
	1972	36	41	16	7
	1973	23	62	13	2
	1974	25	53	18	4
	1975	41	52	7	0
Streptomycin	1956	0	0	2	98
	1970	15	45	20	20
	1972	7	42	30	21
	1973	10	58	22	10
	1974	8	54	24	14
	1975	13	55	25	7
Sulphonamides	1956	2	2	4	92
	1970	12	32	18	38
	1972	5	41	29	25
	1973	10	58	22	10
	1974	13	48	21	18
	1975	14	51	26	9
Chloramphenicol	1956	0	0	0	100
	1970	0	3	2	95
	1972	0	1	3	96
	1973	0	8	19	73
	1974	0	4	30	66
	1975	0	0	8	91
Ampicillin	1970	1	20	15	64
	1972	0	12	18	70
	1973	0	24	35	31
	1974	0	20	48	32
	1975	2	16	51	31
Neomycin	1970	1	1	5	93
	1972	0	1	1	98
	1973	0	5	7	88
	1974	1	0	7	92
	1975	1	2	5	92
Furazolidone	1970	1	3	4	92
	1972	1	1	6	92
	1973	0	4	13	83
	1974	0	0	2	98
	1975	0	0	1	98
Spectinomycin	1972	3	20	26	51
	1973	1	23	34	43
	1974	1	16	31	52
	1975	0	4	43	53
Polymixin, trimethiprim and sodium nalidixate	1972 1973 1974 1975	0	0	0	100

The method of classifying the specimens according to their content of resistant and sensitive *E coli* has been described previously[1].

largely responsible for the finding of *E. coli* organisms resistant to the comparatively rarely used spectinomycin, all of 27 spectinomycin-resistant strains isolated from different pigs also being streptomycin resistant, a probable consequence of the fact that one form of plasmid-determined streptomycin resistance is mediated through an adenylating enzyme that inactivates spectinomycin in addition to streptomycin The increasing incidence of organisms possessing chloramphenicol resistance, most of which was transmissible, during 1970–74 gave some cause for concern The concentration of these organisms in most of the faecal specimens, however, was very low and, fortunately, the increase was not maintained in the 1975 survey. That no *E coli* organisms resistant to trimethoprim, polymixin or nalidixic acid were found in any of the surveys was probably a reflection of the very low degree of exposure of the pig population to these antibiotics.

Table 2 Incidence of transmissible resistance in *E. coli* strains isolated at different times

Resistance studied	Year of isolation	No. of resistant strains examined	% Whose resistance was transmissible
Tetracycline	1970	30	73
	1972	30	60
	1974	50	32
	1975	50	36
Streptomycin	1970		
	1972	28	46
	1974	50	28
	1975	50	40

The failure of prohibition of the growth promotion use of tetracyclines to markedly reduce the amount of tetracycline-resistant *E coli* in the pig population stresses the fallacy of assuming that the ecological changes brought about largely by the persistent and widespread use of antibiotics can be reversed simply by resorting to a policy of withdrawal. It serves also to emphasise the need of strict adherence to the main recommendation of the Swann Report[4] that antibiotics commonly used in human and veterinary medicine should not be used as feed additives for growth promotion so that such undesirable occurrences can be avoided in the future.

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Genetic transfer of nitrogenase–hydrogenase activity in *Rhodopseudomonas capsulata*

THE transfer of nitrogen fixation (*nif*) genes to bacterial mutants defective in this capacity (*nif*$^-$) by transduction[1] and conjugation[2] was first observed in 1971 with *Klebsiella pneumoniae*. The investigations cited and the subsequent demonstration[3] that *nif* genes can be transferred from *K. pneumoniae* to *Escherichia coli* have been widely regarded as harbingers of the molecular biology of the nitrogen fixation process. We now report a new system of interest in this connection, namely, the transfer of *nif* genes in the non-sulphur purple photosynthetic bacterium *Rhodopseudomonas capsulata*. Genetic transfer in this organism is mediated by a filterable vector of small size and unknown nature, the "gene transfer agent" (GTA), discovered by Marrs[4].

Mutants of *Rps. capsulata* unable to fix N_2 were isolated from wild type strain B10 (obtained from Dr B. Marrs; see ref. 4) as follows. Cells were grown photosynthetically (anaerobically) at about 30 °C in a synthetic medium containing DL-malate and 0.1% ammonium sulphate as the carbon and nitrogen sources, respectively (medium RCVBN described in ref. 5). A culture in the late exponential growth phase was treated with N-methyl-N′-nitro-N-nitrosoguanidine (100 μg ml^{-1} for 30 min) and the mutagen was then removed by washing the cells several times by centrifugation. The cells were resuspended in the synthetic medium, incubated for about 18 h anaerobically in darkness, and then illuminated to permit growth and segregation of mutant phenotypes. After the early stationary phase was reached, cells were transferred into synthetic medium from which $(NH_4)_2SO_4$ was omitted, and the anaerobic culture incubated under illumination until formation of gas bubbles was evident (H_2 formation occurs only after all exogenous ammonium sulphate has been used). To enrich for *nif*$^-$ mutants, penicillin G was added (10 U ml^{-1}) and the culture was illuminated anaerobically in a GasPak jar (atmosphere: $N_2+H_2+CO_2$) for 14 h; excess penicillinase was then added and the suspension was left to stand for 1 h at room temperature. Mutants with the *nif*$^-$ phenotype were sought by plating samples on synthetic medium agar containing a decreased concentration of $(NH_4)_2SO_4$ (0.01%); the plates were incubated anaerobically (GasPak jars) in the light. In these conditions, the final diameter of putative *nif*$^-$ colonies is considerably smaller than that of *nif*$^+$ colonies (able to use N_2) and the former can consequently be easily identified. Several isolates obtained in this way were unable to to use N_2 as a nitrogen source for growth and we consider here the properties of five of them (Table 1). Appropriate tests showed that the mutants retained identifiable characteristics of the parental strain, such as photosynthetic growth capacity and persistence of a specific bacteriophage associated only with this strain[6].

Tests for the presence of nitrogenase in mutants W12, W15 and W16 by the sensitive acetylene reduction assay[7] gave negative results (using cells starved for nitrogen or grown with nitrogen sources that do not repress nitrogenase synthesis). These strains cannot use N_2 for growth, but in common with the B10 parental strain can utilise ammonium sulphate, urea, glutamate, glutamine, aspartate, proline, ornithine and thymine as nitrogen sources. Although mutants W11 and W13 show the *nif*$^-$ phenotype, nutritional and genetic experiments indicated that the lesions in these strains are different, and probably associated with defects in synthesis or metabolism of glutamine or glutamate. The data in Table 1 show that GTA produced by cultures of the *nif*$^+$ B10 (parental) strain can be accepted by the *nif*$^-$ mutant strains, resulting in 'transferants' which have regained the capacity to fix N_2. The second column (control) shows the frequency of spontaneously occurring *nif*$^+$ revertants in the mutant populations; the last column shows the greatly increased number of *nif*$^+$ colonies seen after the mutants are treated with GTA obtained from B10 cultures.

Table 1 Conversion of *nif*$^-$ *Rps. capsulata* mutants to *nif*$^+$ phenotype by GTA from wild type B10 cells

Nif$^-$ mutant	*Nif*$^+$ colonies per plate Control	 +GTA
W11	3	917
W12	1	298
W13	1	1,320
W15	4	1,012
W16	6	450

GTA activity was assayed as described by Wall *et al.*[6], essentially using procedures developed by Marrs[4] and Solioz *et al.*[13]. GTA filtrates (0.3 μm Millipore filters) were obtained from early stationary phase cultures of *Rps. capsulata* strain B10 grown photosynthetically in a medium consisting of 0.3% Difco yeast extract, 0.3% Difco Bactopeptone, 2 mM $CaCl_2$ and 2 mM $MgSO_4$ (YPS medium); appropriate controls showed that the filtrates did not contain viable cells. The mutants were also grown photosynthetically in YPS medium; cells were collected in the early stationary phase and resuspended in the original volume of buffer composed of 10 mM Tris-HCl (*p*H 7.8), 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaCl and bovine serum albumin (500 μg ml^{-1}). To test the effect of GTA, equal volumes of filtrate and recipient cell suspension were mixed and the resulting suspension was incubated semi-aerobically in darkness for 1 h at 34 °C. Aliquots of 0.2 ml were then plated as an overlay (in the buffer noted + 0.3% Wilson Ionagar) on an underlay of RCVBN synthetic medium, modified by omission of ammonium sulphate, solidified with 1.2% Ionagar. The plates were placed in anaerobic jars containing N_2 (GasPak system) which were allowed to stand at room temperature for 2 h before incubation under illumination at 30–33 °C. Control values are numbers of spontaneous *nif*$^+$ revertants observed per $3\text{–}4\times10^8$ cells plated. Values under +GTA are numbers of *nif*$^+$ colonies observed per 0.1 ml of B10 filtrate used (or per $3\text{–}4\times10^8$ recipient cells plated after incubation with B10 filtrate). The numbers of transferants observed in such experiments vary considerably from day to day, apparently because of slight differences in conditions affecting the physiological states of donor and recipient cells[6,13]

The possibility that strains W12, W15 and W16 have the same *nif*$^-$ mutation was tested by examining the capacities of filtrates from cultures of each mutant to restore the *nif*$^+$ character to cells of the other strains. Table 2 shows that filtrate from a particular mutant does not have activity with cells of the same strain. GTA from strain W12 gave *nif*$^+$ recombinants with cells of W15 and W16, and similarly, GTA from W15 restored the *nif*$^+$ trait in strains W12 and W16. We conclude that the mutations in the three strains are not identical. Although mutant W16 accepts GTA from strains W12 and W15, it is inactive as a GTA donor. The apparent inability of W16 to

Table 2 Recombination between *nif*$^-$ mutants

Culture filtrate	Recipient cells W12	 W15	 W16
W12	−	+	+
W15	+	−	+
W16	−	−	−

Procedures for growth of cells, preparation of filtrates and GTA tests were as described for Table 1. +, *nif*$^+$ recombinants observed in numbers significantly greater (> tenfold) than the spontaneous reversion rate. −, Number of *nif*$^+$ colonies approximately the same as observed with recipient cells not treated with culture filtrate (that is, spontaneous reversion rate).

produce GTA was checked using another assay, namely, capacity to produce GTA required for restoration of photosynthetic growth competence to a non-photosynthetic white mutant of *Rps. capsulata*[6]. GTA preparations from W12 and W15 showed activity in the latter test, but filtrates from W16 did not. The peculiarities of W16 in this respect are still unexplained and are under investigation.

Typical purple bacteria produce large quantities of molecular hydrogen during photoheterotrophic growth on organic acids (for example, malate) when certain amino acids are used as nitrogen sources (for example, glutamate)[8]. Addition of ammonia, the first stable product of N_2 fixation, causes repression of synthesis of the H_2-evolving system of *Rhodospirillum rubrum*[9], and this is also observed with *Rps. capsulata.* In contrast to the parental B10 strain used in the present studies, *Rps. capsulata* mutants W12, W15 and W16 are unable to produce H_2 during photosynthetic growth in malate+glutamate medium (on occasion, a small quantity of H_2 is observed with mutant W12, which is also leaky with regard to fixation of N_2). All the *nif*$^+$ spontaneous revertants and transferants of these mutants tested so far, however, showed active production of H_2. These observations strongly support the suggestion[10] that in photosynthetic bacteria, nitrogenase and H_2-evolving hydrogenase activities are catalysed by the same enzyme complex. Although other explanations are conceivable (for example, that the two activities in question are attributable to different enzyme systems that are coordinately regulated) we consider that the data available are most plausibly interpreted on the basis of dual functions of the nitrogenase complex.

Studies with cell-free preparations from various N_2-fixing bacteria have revealed that nitrogenases can evolve H_2 by an energy-dependent mechanism[11,12]; photoproduction of H_2 by non-sulphur purple bacteria appears to be the most clear-cut example where such H_2 formation occurs as a normal and major physiological process. The latter is believed to represent a regulatory device that enables the cell to dispose of reducing power and adenosine triphosphate when these are generated in excess of biosynthetic demands[8]; that is, a device that facilitates energy idling. It is worth noting that use of H_2 as an electron donor for photoautotrophic growth apparently is mediated by a different hydrogenase system. This is indicated by the fact that *Rps. capsulata* mutants W12, W15 and W16 grow readily, photosynthetically, with 0.1% $(NH_4)_2SO_4$ as the nitrogen source and H_2+CO_2 as the sole sources of reducing power and carbon, respectively.

Solioz *et al.*[13] found that GTA can be used to demonstrate linkage between genes for carotenoid biosynthesis and those involved in production of bacteriochlorophyll in *Rps. capsulata.* This development and the findings reported here indicate that GTA can be exploited for more refined analysis of the molecular biology of the nitrogenase–hydrogenase system.

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Characterisation of a rotavirus

ACUTE gastroenteritis is one of the most common causes of illness in children, calves and piglets, and causes severe economic loss in domestic animals Although some outbreaks of gastroenteritis are associated with bacterial pathogens, particularly *Escherichia coli*, no known pathogens had been isolated from a significant proportion of outbreaks until recently, when viruses with a distinctive morphology and antigenic similarity were found associated with diarrhoea in piglets, calves and children[1,2]. These viruses are major pathogens and it has also been shown that viruses isolated from man are infectious for the pig[3]. Initially, these viruses were described as reovirus-like[4] but subsequently the names rotavirus[5] and duovirus[6] were proposed. They are morphologically and antigenically distinct from both the reoviruses and orbiviruses[5,7] and also differ from them in some of their physicochemical properties[8].

Studies of the structure of rotaviruses have been confined to the nucleic acid of the calf virus and the related virus of epizootic diarrhoea of infant mice (EDIM). Both viruses were shown to contain RNA[9,10], and Welch and Thompson[8] suggested that the calf virus RNA might be double stranded The results of the experiments described here show that calf rotavirus possesses several of the features characteristic of the reo- and blue tongue viruses and should be classified with them[11].

To facilitate purification of the virus and to confirm the type of nucleic acid, secondary calf kidney cell cultures in 40-ounce roller bottles were infected with a strain of tissue culture adapted calf rotavirus[12] and maintained with Earle's medium containing 2% foetal calf serum and either ^{3}H-uridine or ^{3}H-thymidine. A total of 100 µCi isotope was added to each bottle over a period of 3 d The cells and maintenance medium were frozen and thawed three times, the cell debris removed by centrifuging at 2,000*g* for 5 min and the virus purified by the following method.

The virus was pelleted by centrifuging at 40,000*g* for 1 h, resuspended in 0.1 M Tris, *p*H 7 6, and the insoluble debris

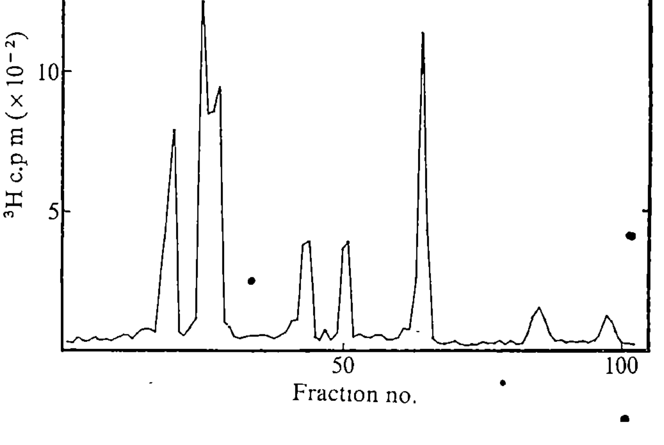

Fig. 1 Separation of the different segments of the ^{3}H-uridine-labelled RNAs of calf rotavirus by polyacrylamide gel electrophoresis in 5% gels for 24 h at 3.5 mA per gel The molecular weights were estimated by reference to the migration of ^{14}C-adenine-labelled reovirus RNAs in the same gel Arrows indicate the positions of the reovirus RNAs: (1) 2 6–2.8 $\times 10^6$; (2) 1 5–1 6 $\times 10^6$; (3) 0 9 $\times 10^6$; (4) 0 8 $\times 10^6$; (5) 0 6 $\times 10^6$ (ref. 14).

removed at 2,000*g*. The supernatant fluid, which contained a large number of rotavirus particles, was mixed with 0.5% sodium deoxycholate and centrifuged at 40,000*g* for 1 h in a 20-ml sucrose gradient (15–45% in 0.1 M Tris, 0.1 M NaCl, *p*H 7.6) layered over a 1-ml cushion of saturated CsCl solution. Fractions (1 ml) were collected and assayed for radioactivity. The cultures which contained ^{3}H-thymidine gave a band of radioactivity at the caesium chloride–sucrose interface with the remainder of the radioactivity at the top of the gradient. The ^{3}H-uridine-labelled cultures also gave a band of radioactivity at the CsCl–sucrose interface, but in addition to the radioactivity at the top of the gradient, there was also a peak at about 500S. This peak contained the major part of the infectivity, and electron microscopical examination showed the presence of a large number of rotavirus particles confirming Welch's observation that the virus contained RNA[9]. Very few particles were found elsewhere in the gradient.

The relative sedimentation coefficient of the virus was determined by cosedimenting a mixture of the ^{3}H-labelled virus and unlabelled reovirus in a sucrose gradient. Well separated peaks of radioactivity and ultraviolet absorbing material were obtained. Using a value of 630S for reovirus, a relative value of 500S was calculated for the rotavirus.

The buoyant density of the virus was determined by centrifuging a mixture of ^{3}H-rotavirus and ^{32}P-Maus–Elberfeld virus at 60,000*g* for 17 h in 5 ml preformed CsCl gradients (density range 1.30–1.40 g ml^{-1}). The distribution of radioactivity showed that the two viruses sedimented as sharp peaks with densities of 1.36 and 1.33 g ml^{-1} respectively. The value for the Maus–Elberfeld virus is in close agreement with that found by Rueckert and Schäfer[13], and the density determined for the rotavirus agrees with the value found by Welch[9]. When the rotavirus suspension was centrifuged in CsCl without first treating with deoxycholate, two distinct bands at densities of 1.36 and 1.38 g ml^{-1} were obtained which contained rotavirus particles with and without their outer capsid layer, respectively (J.C.B. and G.N.W., unpublished). A similar result was obtained when the peak of ^{3}H-uridine radioactivity located at the CsCl–sucrose interface in the purification procedure described here was centrifuged in a CsCl gradient.

The RNA was extracted from the purified ^{3}H-rotavirus by diluting the fractions from the 15–45% sucrose gradient with 5 volumes 0.1 M Tris, *p*H 9.0, containing 0.5% SDS, and shaking with phenol. All the radioactivity was found in the aqueous phase, which was mixed with ribosomal RNA prepared from BHK21 cells and the mixture of RNAs precipitated with 2 volumes ethanol at −20 °C. To determine whether rotavirus RNA was single or double stranded, the precipitate was dissolved in 2 ml 0.04 M phosphate, *p*H 7.6 and 1 ml was incubated with 0.01 μg pancreatic RNase at 20 °C for 15 min. The untreated and RNase-treated samples were then centrifuged at 35,000*g* for 18 h in separate 5–25% sucrose gradients in 0.1% SDS–0.1 M acetate, *p*H 5.0. The distribution of radioactivity was the same in both gradients with a broad peak at 11–16S but the A_{260} profiles showed that the single-stranded ribosomal RNA had been hydrolysed in the sample treated with RNase. The broad peak of radioactivity suggested that the virus RNA was heterogeneous and this was confirmed by electrophoresis in 5% polyacrylamide gels for 24 h at 3.5 mA per gel, when eight peaks of radioactivity were obtained (Fig. 1). To enable their molecular weights to be calculated, a mixture of the RNAs of ^{3}H-uridine rotavirus RNA and ^{14}C-adenine reovirus, type 3, was electrophoresed in the same conditions and 1-mm gel slices counted for each isotope. Using the molecular weights given by Martin and Zweerink[14] for the reovirus RNAs, values of 2.2, 1.9, 1.7, 0.9, 0.8, 0.5, 0.3 and 0.2×10^6 were calculated for the rotavirus RNAs. The relative radioactivity of each peak indicated that the two peaks with molecular weights of 1.9 and 0.5×10^6 consisted of two and three or four species respectively. This means that the rotavirus RNA consists of 11 or 12 segments with a total molecular weight of $11\times10^6-12\times10^6$.

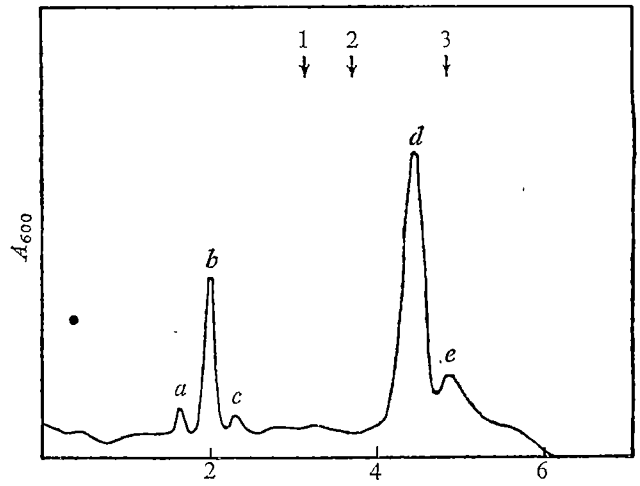

Fig. 2 Separation of the polypeptides of calf rotavirus by polyacrylamide gel electrophoresis in 7.5% gels for 16 h at 5mA per gel. The molecular weights were estimated by reference to the migration of ^{35}S-methionine-labelled VSV proteins in the same gel. Arrows indicate the positions of the marker proteins: (1) 69×10^3; (2) 50×10^3, (3) 29×10^3 (ref 15)

Table 1 Comparison of the properties of reovirus, blue tongue virus and calf rotavirus

Property	Reovirus		Blue tongue virus		Calf rotavirus (present work)	
Sedimentation coefficient	630		550		about 500	
Diameter (nm)	60–75		69		60–65	
Buoyant density in CsCl (g ml^{-1})	1.38		1.36		1.36–1.38	
RNA and proteins	Polypeptide MW×10^{-3}	RNA MW×10^{-6}	Polypeptide MW×10^{-3}	RNA MW×10^{-6}	Polypeptide MW×10^{-3}	RNA MW×10^{-6}
	155	2.5 (2.8)	155	2.7 (2.8)	135	2.2 (2.4)
	140	2.4 (2.5)	110	2.0 (2.0)	122	1.9 (2.2)
	—	2.3 —	100	1.9 (1.8)	96	1.7 (1.7)
	80 and 72	1.6 (1.4)	72	1.3 (1.3)	—	0.9 —
	42	0.9 (0.8)	58	1.1 (1.0)	36	0.8 (0.7)
	38	0.8 (0.7)	38	0.6 (0.7)	30	0.5 (0.5)
	34	0.6 (0.6)	32	0.6 (0.6)	—	0.3 —
			—	0.5 —	—	0.2 —
			—	0.3 —		
Capsid proteins (species)	7		7		5	
Total number of double-stranded RNA segments	10		10		11–12	
Total molecular weight of RNA	14.7×10^6		12×10^6		$11–12\times10^6$	

Figures in parentheses indicate the double-stranded RNA molecular weight (MW) required to code for each polypeptide, assuming a polypeptide to double-stranded RNA ratio of 18 (ref 14)

Data on reo- and blue tongue viruses were obtained from refs 11 and 14, respectively

The polypeptides of the virus were examined by polyacrylamide gel electrophoresis. Purified virus was digested at 37 °C for 1 h with 8 M urea, 1 0% SDS, 0 1% mercaptoethanol, dialysed against 0.5 M urea, 0.1% SDS, 0.1% mercaptoethanol in 0.01 M phosphate, *p*H 7.2, and the mixture of polypeptides electrophoresed in 7.5% polyacrylamide gels for 16 h at 5 mA per gel. Staining with 0.5% Coomassie blue revealed the presence of at least two major bands (*b* and *d*) and three minor bands (*a*, *c* and *e*, Fig 2) The two minor bands *c* and *e* were present in variable amounts in different preparations. For an assessment of their molecular weights, a mixture of the rotavirus polypeptides and a quantity of ^{35}S-methionine-labelled vesicular stomatitis virus polypeptides which was insufficient to stain with Coomassie blue was coelectrophoresed in the conditions described above. The gel was stained to ascertain the positions of the rotavirus polypeptides and then cut into 1-mm slices for counting (Fig. 2). The molecular weights of the rotavirus polypeptides were calculated to be 135, 122, 96, 36 and 30×10^3. A possible coding relationship between these polypeptides and the RNAs is shown in Table 1.

Although the calf rotavirus has a smaller sedimentation coefficient and is morphologically distinct from the reo- and blue tongue viruses, the segmented and double-stranded nature of its RNA and the number and molecular weights of the RNA and polypeptides suggest that it should be regarded as a member of the reo- and blue tongue virus group. Its precise relationship to the other members of the group, however, will require extensive cross hybridisation studies of the virus RNAs.

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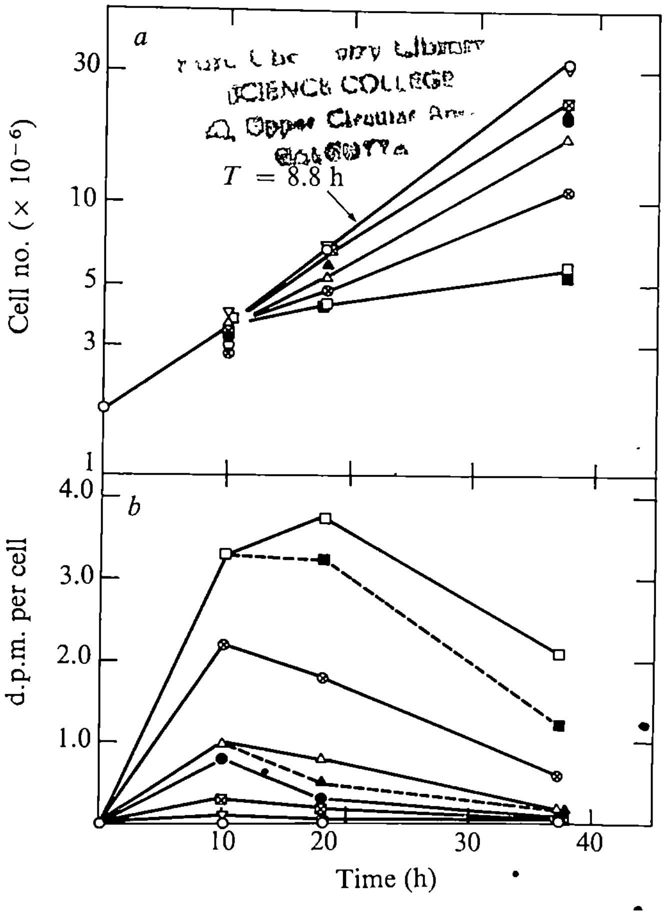
Perturbations in cell cycle progression from radioactive DNA precursors

RADIOACTIVE precursors of DNA have widespread application in eukaryotic cell systems for studies of kinetic phenomena, such as DNA metabolism and cell population growth, and for labelling DNA[1,2] for biophysical or biochemical investigations. Implicit in their use is the assumption that the experimental endpoints are not perturbed by the presence of the labelling isotope(s). But the validity of this assumption requires re-evaluation in the light of reports that the incorporation of radioactive compounds into cellular DNA causes significant biological anomalies, including mutations, chromosome aberrations, cell death[3], growth retardation and strand breaks in the DNA molecule[4] (for reviews see refs 5 and 6). At least one investigator[5] has advocated separate evaluation of the potential perturbations in every experimental system using radioisotope-labelled nucleic acid precursors.

Because the first step in many experiments is lengthy labelling (for example, overnight) with a radioactive DNA precursor, and because our unpublished observations indicate unusual growth behaviour by several mammalian cell lines in the presence of tritiated thymidine (^{3}H-TdR), we investigated the influence of this precursor on the cycle progressions of those mammalian cell lines by high speed flow microfluorometry (FMF)[7,8]. With this technique, the cells in the G_1, S, and G_2 + M phases of the cell cycle can be counted by direct measurement of the DNA content per cell. Representative data obtained with one cell line are presented in preliminary form now because they exemplify growth perturbations that must be considered when studies with radioactive precursors are interpreted.

Monolayer cultures of Chinese hamster fibroblasts (V79-182) were cultured as described before[9,10]. Petri dishes (100 mm, Falcon) were inoculated with equal numbers of V79-182 cells in FST-15 medium (Eagle's basal medium supplemented with antibiotics, 4% NCTC-109, trypsin, glutamine and 15% undialysed foetal calf serum). After overnight incubation at 37 °C to provide exponential populations (doubling time about 9 h), FST-15 was replaced with fresh, warm (37 °C) EM-15 medium (FST-15 without NCTC-109 or trypsin)

Fig. 1 Cell proliferation (*a*) and ^{3}H d.p m. per cell (*b*) of V79 fibroblasts after continuous incubation in the presence of various concentrations of ^{3}H-TdR. Fresh growth medium containing ^{3}H-TdR was added to exponentially growing cultures at zero time: ○, control (no ^{3}H-TdR); ▽, 0 025 μCi ml^{-1}; crossed square, 0.075 μCi ml^{-1}; △, 0.25 μCi ml^{-1}; ▲, 0.25 μCi ml^{-1}, removed after 10 h; crossed circle, 0.5 μCi ml^{-1}; □, 1.0 μCi ml^{-1}, ■, 1.0 μCi ml^{-1}, removed after 10 h; ●, 20 μCi ml^{-1}, flash exposure (20 min) *T*, mean doubling time of control populations.



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containing ^{3}H-methyl-thymidine (6.7 Ci mmol^{-1}, New England Nuclear Corp.) at concentrations varying from 0.025 to 1.0 μCi ml^{-1} for continuous labelling or at 20 μCi ml^{-1} (15 Ci mmol^{-1}, New England Nuclear Corp.) for flash labelling. After various periods of incubation at 37 °C, the radioactive medium was aspirated from a group of dishes and the cells were washed twice with 10 ml of FST-15 medium supplemented with non-radioactive (5 μg ml^{-1}) thymidine and then trypsinised into suspension. Each sample was divided into three aliquots: the first was assayed for cell number with a haemocytometer; the DNA radioactivity in the second aliquot was determined by liquid scintillation spectrometry after precipitation of the DNA with perchloric acid; the third aliquot was prepared for DNA analysis by FMF according to the procedures of Van Dilla *et al.*[7,8,11,12]. These procedures determine the distribution of cells throughout the cell cycle by measuring the DNA content of each individual cell and then constructing a spectrum of cellular DNA contents for the entire population[7,8,13]. The DNA within the cell is stained with a fluorescent dye such as acriflavine, and the cell population is passed through a laser beam that excites the dye. The resulting fluorescence from each cell is proportional to the amount of fluorescent dye bound to the DNA within the cell, and thus is proportional to the DNA content of that cell[7,8]. At least 50,000 cells were assayed for every DNA spectrum given in the figures. Some FMF measurements were made with the fluorometer at the Los Alamos Laboratory of the University of California[7,8]; the others were made with a similar instrument at the Lawrence Livermore Laboratory of that university.

Incorporation of ^{3}H-TdR affected the growth of cells

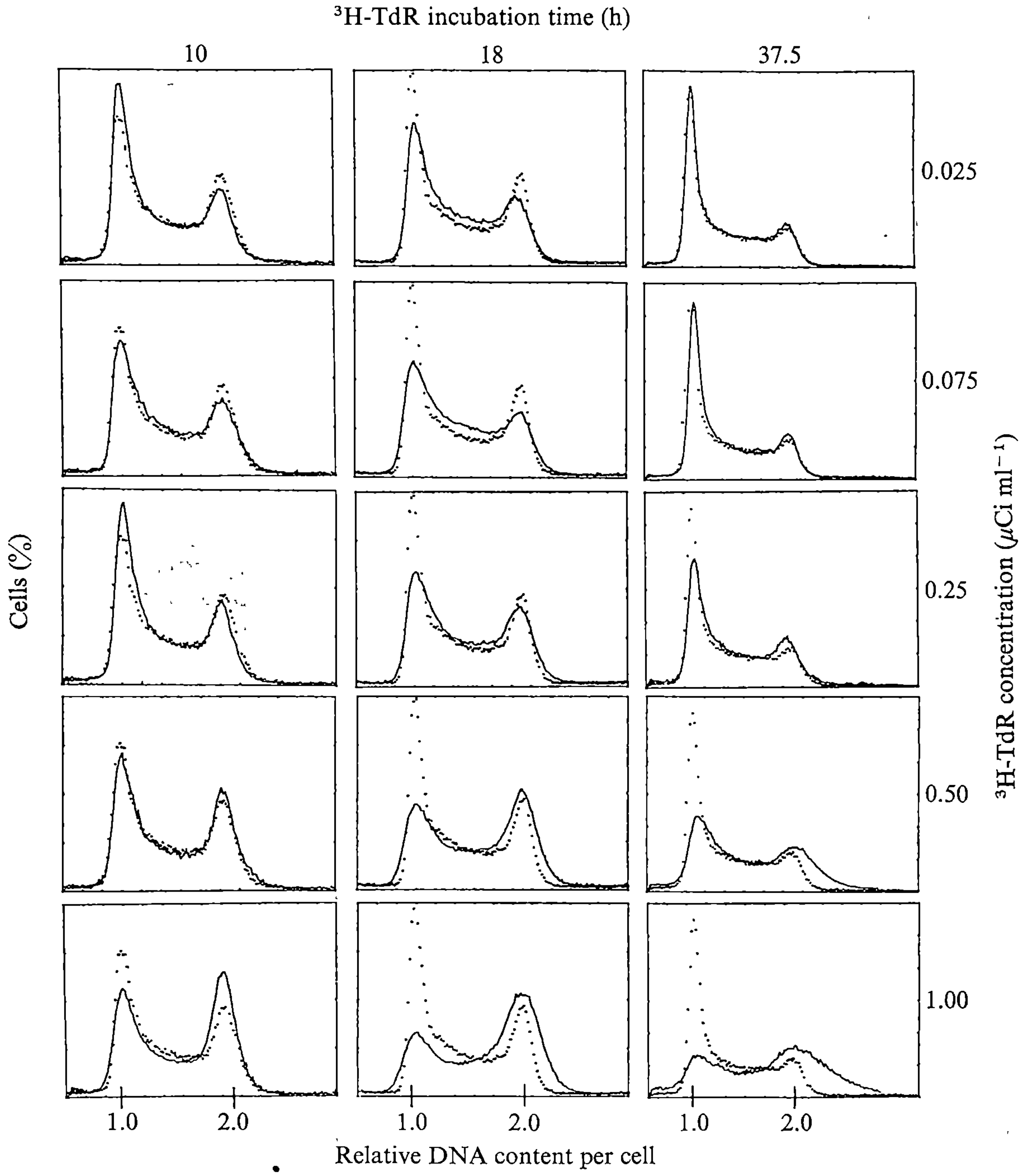

Fig. 2 Distribution of DNA contents of V79 fibroblasts as a function of incubation time with graded concentrations of ^{3}H-TdR. ^{3}H-TdR, at the desired concentration in fresh growth medium, was added to the exponentially growing cultures at zero time Samples of ^{3}H-TdR-treated cells (——) and control cells (. . . .) were prepared for FMF after various incubation times. The positions of the G_1 and the G_2+M peaks are represented by 1.0 and 2.0 relative DNA contents, respectively, and the area between the peaks represents the S-phase cells.

(Fig. 1*a*) even when the precursor was depleted substantially after one cell generation (Fig. 1*b*). The cell counts taken after 10 h of incubation were statistically the same for all samples by the *F* test. But after 18 and 37.5 h (equivalent to more than four doubling times for controls), the growth rates decreased progressively once the concentration of the precursor exceeded 0.025 μCi ml^{-1}. To verify that the precursor was depleted from the medium during the first doubling time, the medium was removed from two of the samples after 10 h and replaced with fresh non-radioactive medium. As expected, this treatment lowered the radioactivity in the cells only slightly and the subsequent growth rates were virtually unchanged (Fig. 1).

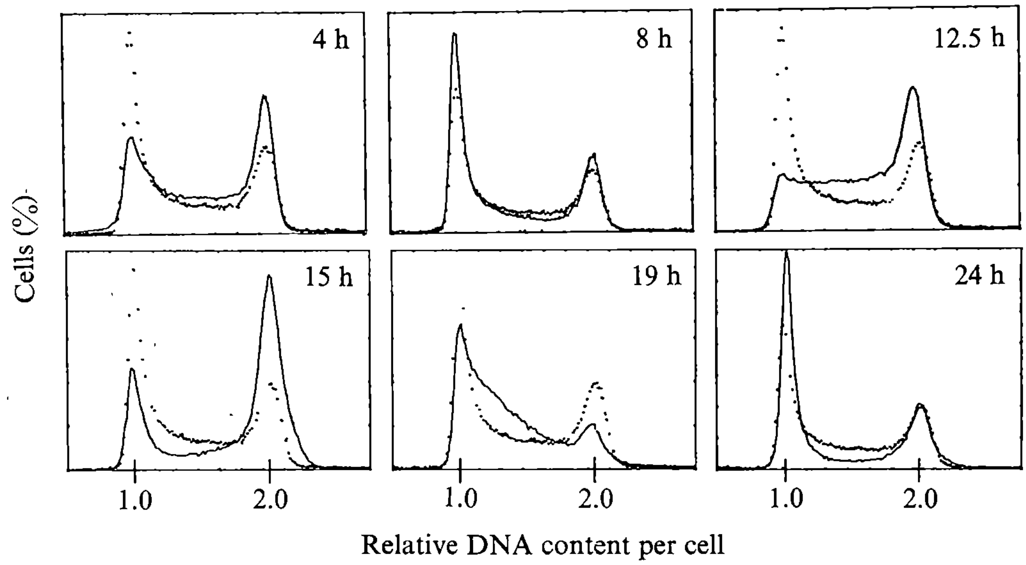

Fig. 3 Distribution of DNA contents of V79 fibroblasts as a function of time after exposure to a flash label (20 min) of ^{3}H-TdR (20 μCi ml^{-1}, 15 Ci mmol^{-1}). After the 20-min incubation with ^{3}H-TdR, the medium was aspirated and replaced, after several rinses, with fresh, warm growth medium. ^{3}H-TdR-treated cultures (——) were fixed after various incubation times and compared to untreated controls (· · · ·). The positions of the G_1 and the G_2+M peaks are represented by 1.0 and 2.0 relative DNA contents, respectively. The area between the peaks represents the S phase cells.

The distribution of DNA contents per cell for control samples (Fig. 2, dotted lines) at 10 h and 18 h were those expected for rapidly dividing, exponential populations[7,8,11–13]; the change at 37.5 h indicates a greater proportion of cells with a G_1 content as the confluent population approached the plateau phase of growth. But some of the DNA profiles for cells incubated in the presence of ^{3}H-TdR (Fig. 2, solid lines) exhibited abnormalities, the magnitude and time of appearance of which depended on the level of radioactivity. For cell populations incubated with high levels of ^{3}H-TdR (0.5 and 1.0 μCi ml^{-1}), the most striking abnormality was the presence of an unusually large G_2 + M peak, indicating a cycle block. This was noticeable after 10 h and persisted until the last sampling time of 37.5 h. Although G_2 cells cannot be distinguished from M cells by fluorometry, we believe that most cells blocked in G_2 + M were actually G_2 cells because microscopic examination did not reveal the presence of unusually high numbers of mitotic cells. The possibility that late-S cells contributed to the G_2 + M peak cannot be ruled out. The shape of the G_2 + M peak was also abnormal. At 37.5 h, for example, the peak for the 1.0 μCi ml^{-1} sample had a long tail, which indicates the presence of cells with DNA complements greater than normal G_2 or M cells (4C DNA). Concomitantly, the G_1 peak in this sample contained cells with less DNA than G_1 controls. Other indications of the severity of the effects of the highest level of radioactivity were the presence of many giant cells and some necrotic cells in the 1.0 μCi ml^{-1} samples at 18 h and 37.5 h.

With low ^{3}H-TdR concentrations (up to 0.075 μCi ml^{-1}), the block in the G_2 + M phase of the cycle was transient. The cells had overcome this block by 10 h, and the resulting semi-synchrony produced populations with more cells in G_1 and early S than in control populations (Fig. 2). This cell cycle perturbation was even more evident one generation later, at 18 h, but by 37.5 h the DNA profiles were again like those of the control populations..

Because flash labelling with ^{3}H-TdR of high specific activity is a common experimental technique, we also did a 20-min flash-labelling experiment using ^{3}H-TdR at 20 μCi ml^{-1}. Four hours after flash labelling, a large fraction of the cell population had a G_2 + M DNA content (Fig. 3). After another 4 h, the cells had overcome the block and progressed into G_1 (Fig. 3) in a semi-synchronous fashion. This synchrony evidently persisted for at least two cell generations (Fig. 3). Exposure to non-radioactive thymidine at the same concentrations as used for ^{3}H-labelled cells (1.33 μM), either pulsed or continuously, did not change the DNA distributions from those found with control populations (data not shown).

The perturbations in growth of V79 cells caused by ^{3}H-TdR in these experiments are unequivocal. Perturbations analogous to those demonstrated here, but with different severity, were observed for Chinese hamster ovary fibroblasts, L5178Y murine leukaemic lymphoblasts, Chang's liver cells (LICH) and human skin fibroblasts (WI-38). The most prominent effect on the DNA profiles for all these cell types was the appearance of a G_2 + M block, the severity and length of which depended on the level of incorporated radioactivity. This expression of damage from internal irradiation by ^{3}H-TdR is similar to the G_2 block exhibited by cells that have been irradiated with external sources of ionising radiation[11,12]. Strict comparison of the dose from an external source with the internal β particle of ^{3}H-TdR is not possible without further experimentation because of the localisation of the tritium decay within the cell nucleus and the chronic nature of the β irradiation, which is complicated by both the rate of uptake of ^{3}H-TdR and the rate of cell division. In these experiments, however, a rough estimate of the dose received during the first generation by cells grown in medium with ^{3}H-TdR at 0.5 μCi ml^{-1} for that time is 200 rad (one ^{3}H-TdR disintegration $\simeq$ 0.35 rad, refs 3 and 4).

Many investigators have emphasised the need for caution when radioactive DNA precursors are used in experiments because of their deleterious effects on cells (see review by Wimber[5]). Experimental results will be misleading if the endpoints measured are affected by incorporated radioactivity. For example, DNA repair mechanisms in externally irradiated eukaryotic cells should be studied with the lowest possible levels of incorporated radioactivity in order to permit precise evaluation of specific radiation responses[14,15]. Our data emphasise the need for caution in the use of radioactive DNA precursors because perturbations in the cell cycle occur at or below the precursor levels in general use in cell biology. Simple cell counting may not be sufficient to determine alterations in cycle progression caused by labelling. For example, in our experiments, cell counts taken 10 h (that is, one cell doubling) after the addition of ^{3}H-TdR to the medium indicated that growth had occurred normally, yet some of the DNA profiles already showed abnormal cell cycle distributions. Thus, it is clear that when radioactive DNA precursors are used as labels in experiments, accurate interpretation of the experiments will require determination of the exact locations of radioisotope-induced blocks in the cell cycle, the relative effects of DNA precursors labelled with different isotopes (for example, ^{14}C-TdR, ^{125}I-uridine), the relative effects of uniform and flash labelling, and the differences in sensitivities of different cell types.

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Replication of human KB cell DNA after infection by adenovirus type 12

Lytic infection of human KB cells by adenovirus type 12 (ad 12) results in the early inhibition of host cell DNA synthesis[1]. We have looked at some aspects of cell DNA replication after infection of KB cells by ad 12. We observed that the ligation of the Okazaki fragments is slower in cells affected by this virus We also observed that the inhibition of cell DNA synthesis by this virus is not a random process and that certain classes of DNA, distinguished by their higher buoyant density and by their high reiteration frequency, are selectively synthesised.

Human KB cells were prelabelled with ^{14}C-thymidine (^{14}C-TdR) for 15–24 h and then infected with ad 12 or mock infected. After 8 h of infection cells were pulse labelled with ^{3}H-TdR for 15 min and then chased for the indicated times. The cells were then lysed and their DNA was analysed by alkaline sucrose gradient centrifugation[2]. As Fig 1 shows, most label in a 15-min pulse is incorporated into lower molecular weight DNA in both infected and control cells. This is consistent with the Okazaki[3] model of DNA replication observed also in other cell types[4].

The small fragments were chased into higher molecular weight DNA. The process was essentially completed by 4 h in the mock infected cells. This was shown by the cosedimentation of pre-existing ^{14}C-DNA with the newly synthesised ^{3}H-DNA Chain elongation was slower in the infected cells (Fig. 1).

In a randomly growing culture of cells the buoyant density of the newly synthesised DNA is the same as that of pre-existing DNA (Fig. 2*a*). Cells were prelabelled with ^{14}C-TdR and infected or mock infected After 8 h of infection, they were pulse labelled with ^{3}H-TdR for 1 h with or without chasing for up to 6 h (no difference was observed with or without chasing). The cells were lysed and treated with Pronase and then centrifuged to equilibrium in neutral CsCl gradients. As Fig. 2*b* shows, the density of the newly synthesised DNA is higher than that of pre-existing DNA in the infected cells. The higher buoyant density is not due to single strandedness of the newly synthesised DNA or to the presence of single-strand tails in the infected cell DNA since the shift is still observed when DNA is treated with S_1 nuclease[7,8] before its centrifugation in CsCl gradients (Fig 2 inset). The observed shift in density is more pronounced if deoxycytidine is used as label (Fig. 2*d*). This is expected if the change in density is due to higher GC content of the newly synthesised DNA in the infected cells.

Table 1 Hybridisation of newly synthesised KB cell DNA to immobilised viral DNA

Sample	Input (c.p.m.)	Bound (c p.m)	% Bound
Empty filters	5,305	59	1.1
Viral DNA	5,305	3,240	61.1
Control (heavy side)	85,638	525	0.61
Control (light side)	50,128	301	0 6
Infected (9 h heavy side)	13,217	216	1.6
Infected (9 h light side)	11,059	92	0.83

Cells prelabelled with ^{14}C-TdR were infected or mock infected and pulse labelled with ^{3}H-TdR and centrifuged as described in Fig. 3. Samples (20 µl) were taken to determine the ^{14}C-labelled radioactive peak. The gradient of DNA from infected cells was then divided so that 54% and 46% of the ^{14}C-label from the left and right sides (referred to as heavy and light sides, respectively) were pooled. The percentages for the control gradient were 63 and 37, respectively. The pooled fractions were dialysed extensively against 0.1 × SSC. Hybridisation to cold viral DNA immobilised on nitrocellulose membrane filters (1 µg per filter) in duplicate was at 66 °C for 24 h. Radioactivity in each filter was then determined after extensive rinsing to remove the unbound DNA.

The shift in density appears by 7 h after infection (Fig 3) and increases further with time It is interesting that the percentage of inhibition of DNA synthesis (Fig 3 inset) also increases with time.

The buoyant density of ad 12 DNA is slightly higher than that of host cell DNA (cellular DNA, $\rho = 1.701$; ad 12 DNA, $\rho = 1.709$)[1]. The observed shift in density, however, cannot be due to contamination with newly synthesised viral DNA as shown by Table 1. No viral DNA is detectable by hybridising the newly synthesised DNA pooled from the heavy or light side of CsCl gradients to unlabelled viral DNA immobilised on filters. This is consistent with the earlier report that the onset of viral DNA synthesis is approximately 12 h after infection in these cells[5].

The genome of most higher organisms contains classes of DNA with different reiteration frequencies[6]. To determine whether infection affects the synthesis of classes of DNA with different degrees of repetition, the kinetics of reassociation of newly synthesised DNA from both infected and

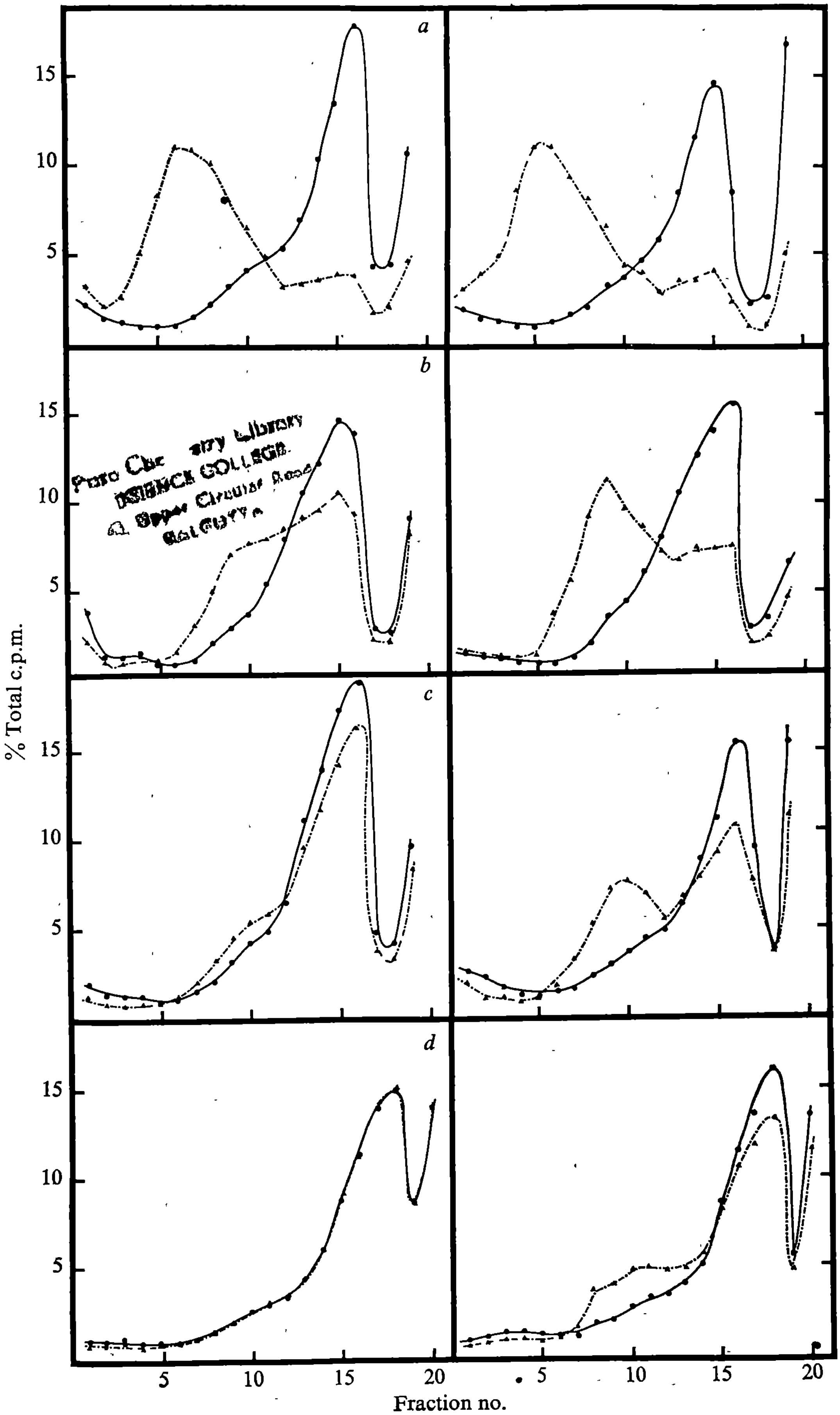

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Fig. 1 Human KB cells, grown on monolayers and prelabelled with ^{14}C-TdR (50 μCi mmol^{-1}, New England Nuclear Corp.) for 15–20 h, were trypsinised and resuspended in infecting medium (Joklik+1% calf serum). About 3×10^6 cells in 1.5 ml were seeded in 25 cm^2 flasks and adsorbed with 3×10^3 virus particles per cell by shaking at 37 °C. Control cells were treated similarly except without the virus. After 90 min of adsorption, 3.5 ml of growth medium (α-MEM, Flow Laboratories) containing 10% serum was added and the cells were incubated at 37 °C. Eight hours after infection, ^{3}H-TdR (5 Ci mmol^{-1}, New England Nuclear Corp.) was added for a 15 min pulse and chased for the indicated times. Centrifugation of cellular DNA in alkaline sucrose gradients was at 20,000 r.p.m. for 6.5 h at 20 °C using an SW 27.1 rotor in a Beckman ultracentrifuge. ●, ^{14}C; ▲, ^{3}H. (Direction of sedimentation from left to right.) Left, control; right, infected; *a*, 0.25 h pulse; *b*, 2.5 h chase; *c*, 4 h chase; *d*, 6 h chase.

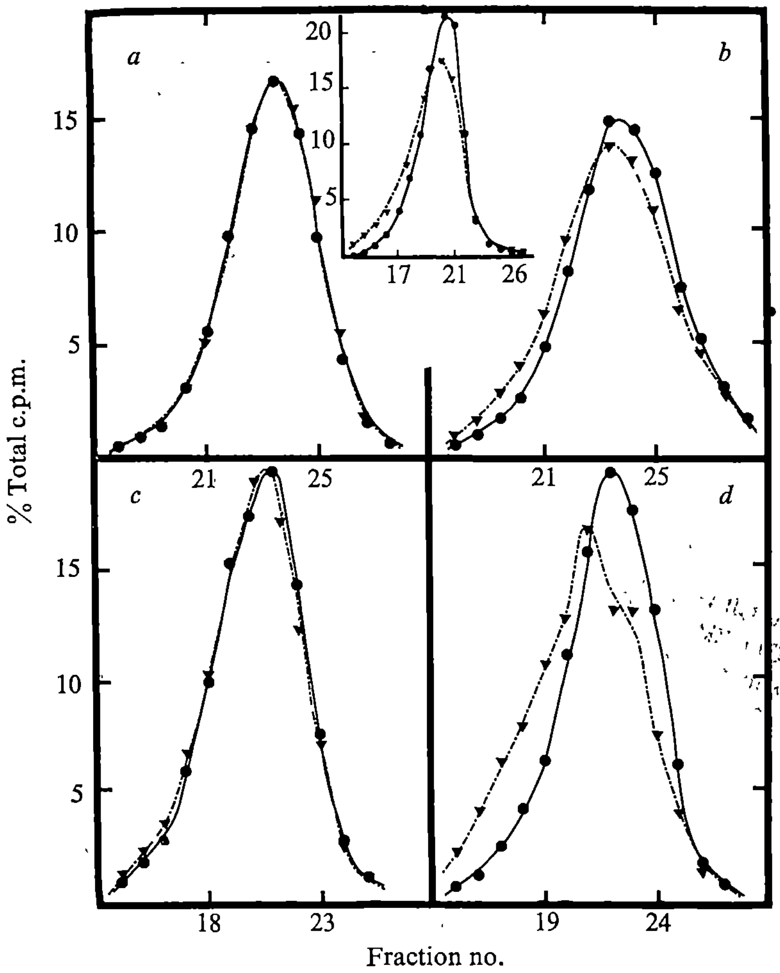

Fig. 2 Cells were prelabelled and infected as described in Fig. 1. They were pulse labelled for 1 h with ^{3}H-TdR and washed and lysed in 4 ml of phosphate buffer (0.1 M NaH_2PO_4, 0.001 M EDTA, 0.1% sodium dodecyl sulphate (SDS), adjusted to *p*H 6 by adding 1 M Na_2HPO_4) and treated with Pronase (1g ml^{-1}) for 30 min at 37 °C. For the inset, DNA was further purified by phenol extraction, dialysing extensively against Tris buffer (0.01 M, *p*H 7.3), and then treating with S_1 nuclease. Tris buffer (1 M, 0.46 ml) containing 10% sarkosyl was added to adjust the final concentration to 0.1 M Tris—1% sarkosyl, *p*H 7.3. DNA was sheared to an average molecular weight of 2×10^7 daltons by passing the solution through a 21-gauge needle. CsCl powder (5.4 g) was added to the solution in a siliconised polyallomer tube and then overlaid with mineral oil. Centrifugation was in a 50 titanium rotor at 40,000 r.p.m. at 20 °C for 65 h. Ten-drop fractions were collected and radioactivity in each fraction was determined after cold TCA precipitation. ●, ^{14}C; ▲, ^{3}H. *a*, control; *b*, infected; *c*, control, labelled with deoxycytidine; *d*, infected, labelled with deoxycytidine. (Direction of sedimentation from right to left.)

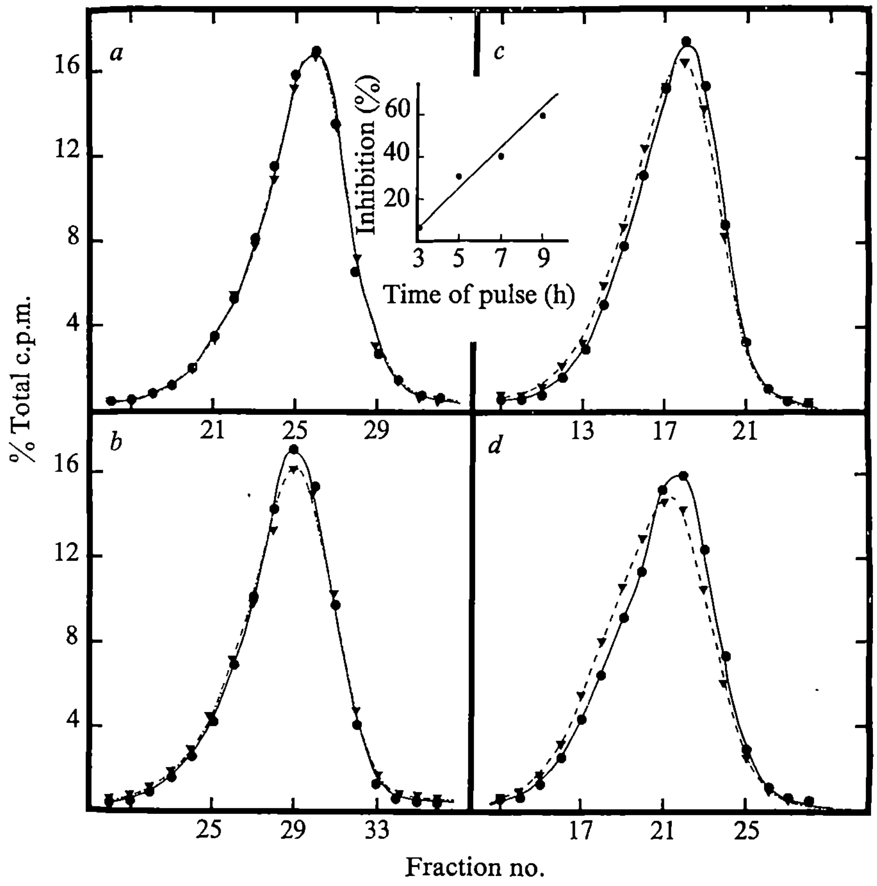

Fig. 3 Cells, prelabelled with ^{14}C-TdR, were infected or mock infected as described in Fig. 1. They were then pulse labelled with ^{3}H-TdR for 30 min at 3(*a*), 5(*b*), 7(*c*) and 9 h(*d*) after infection. DNA was centrifuged to equilibrium in CsCl as described in Fig. 2. Percentage of inhibition of cell DNA synthesis (inset) was determined by comparing the ratio of ^{3}H-DNA to ^{14}C-DNA of the control cells to that of infected cells.

uninfected cells was studied. KB cells were infected with ad 12 and then labelled with ^{3}H-TdR for 2 h at 7 h after infection Control cells were labelled with ^{14}C-TdR. The two were combined and mixed with unlabelled, uninfected cells in order to drive the reannealing reaction by excess cold DNA. The reassociation of denatured DNA was followed using the technique of S_1 nuclease digestion of single-stranded DNA[7,8]. As Fig. 4 shows, the reassociation of DNA follows a multi-component pattern with an apparent break at C_0t of 10. Only 20% of the newly synthesised DNA from uninfected cells reassociates with a C_0t of 10, whereas 30% of the DNA synthesised in the infected cells reassociates with the same C_0t value. Thus, the repetitive sequences represent a greater percentage of newly synthesised DNA in the infected cells.

In human KB cells the DNA synthesised in a short pulse is mostly in the form of small fragments. The conversion of these fragments into high molecular weight DNA is slower in cells infected with ad 12. This slow rate of chain elongation could be due to an inhibition of cell DNA synthesis (inset Fig. 3), since a similar result is obtained when DNA synthesis is inhibited by treating these cells with excess thymidine (our unpublished observation).

Selective inhibition of cell DNA synthesis after infection by ad 12 is the first report of such an effect by an animal virus. The biological significance of this selective inhibition is unknown. Integration of viral DNA into the DNA of these cells has been detected later in infection[9]. It is possible that those classes of DNA which are selectively synthesised are the sites for the integration of viral DNA.

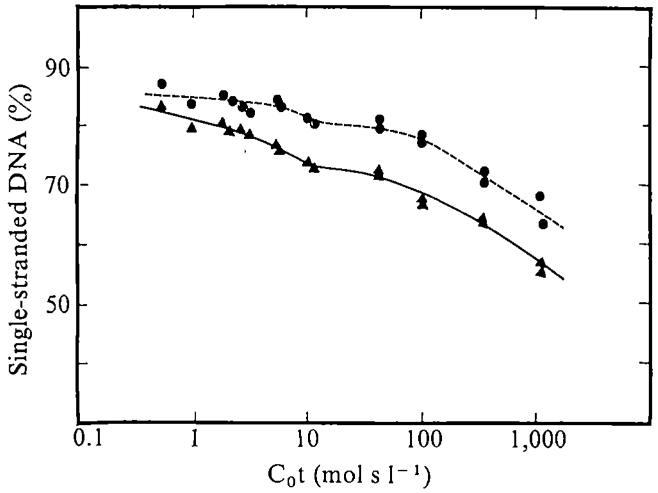

Fig. 4 About 6×10^6 cells were infected or mock infected as described in Fig. 1. After 7 h of infection, the cells were labelled with ^{3}H-TdR for 2 h. Control cells were labelled with ^{14}C-TdR. The two were combined and mixed with 6×10^8 unlabelled cells and lysed in Tris-SDS buffer (0.01 M Tris, 0.1% SDS). After digestion with Pronase (1 g ml^{-1}) at 37 °C, DNA was phenol extracted and ethanol precipitated. After resuspension in distilled water, 1×SSC (0.15 M NaCl, 0.015 M sodium citrate) was added to a final concentration of 0.1×SSC. RNase treatment (50 µg ml^{-1}) was for 30 min at 37 °C. After phenol extraction, the DNA was ethanol precipitated and redissolved in 0.1 M Tris, *p*H 7.3 and dialysed extensively against the same buffer. ^{3}H-DNA was sonicated (the size of sonicated DNA was the same as sonicated ^{14}C-DNA as shown by their cosedimentation in alkaline sucrose gradients) chelex treated, and denatured by boiling. NaCl was added to a final concentration of 0.3 M. Reassociation was in a 2 ml siliconised flask at 67 °C. Samples (50 µl) were taken and treated (untreated as input) with S_1 nuclease in the presence of denatured calf thymus DNA (20 µg ml^{-1}) at 37 °C for 30 min. DNA resistant to S_1 was precipitated with cold trichloroacetic acid (TCA). DNA concentration was 1.45 mg ml^{-1}. Specific activity of ^{3}H-DNA was 1.4×10^4 c.p.m. mg^{-1} and that of ^{14}C-DNA was 4.7×10^3 c.p.m. mg^{-1}. Resistance of denatured DNA to S_1 nuclease was 5% for ^{3}H-DNA and 4% for ^{14}C-DNA. Resistance of non-denatured DNA was 95% for both ^{3}H-DNA and ^{14}C-DNA. ●, ^{14}C, ▲, ^{3}H

The mechanism by which the selective inhibition of cell DNA synthesis is induced is unclear. It is possible that infection results in the selective inhibition of host DNA polymerases which may have specificity with respect to the recognition of their templates. But the direct role of the functional genes of the virus cannot be excluded, especially since no shift in density is observed if the cells are infected with ultraviolet-irradiated ad 12 (M.M.P and S.M., unpublished). Genetic analysis has shown the presence of two[10] or three[11] complementation groups which are necessary for the replication of viral DNA. These could be genes coding for viral specific polymerases. Selective inhibition of cell DNA synthesis could be due to recognition of certain regions of host DNA by such postulated polymerases.

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Are glycoproteins and glycosaminoglycans components of the eukaryotic genome?

THE eukaryotic genome is composed primarily of DNA and a complex and heterogeneous array of proteins. Evidence is rapidly accumulating to suggest that these chromosomal proteins are important in determining structural as well as functional properties of the genome[1–6]. In addition to being implicated in packaging of the cell's genetic information[7–12], chromosomal proteins render limited and defined genetic sequences available for transcription[13–23]. But the specific properties and mode of interaction of genome components remain to be resolved. We present here evidence that several molecular weight classes of chromosomal proteins are glycoproteins. Furthermore, we report the association of glycosaminoglycans, another class of carbohydrate-containing macromolecules, with the eukaryotic genome.

Exponentially growing HeLa S_3 cells were labelled for 48 h with ^{3}H-glucosamine (1 µCi ml^{-1}) and three nuclear preparations were isolated. Nuclei containing intact nuclear envelopes as well as some adhering cytoplasm (crude nuclei) were prepared by osmotically swelling cells in 10 mM KCl, 1.5 mM $MgCl_2$ and 10 mM Tris, *p*H 7.4, followed by lysis in a Dounce type homogeniser. Nuclei stripped of the outer aspect of the nuclear envelope (clean nuclei) were prepared by washing crude nuclei with the non-ionic detergent Triton X-100. Chromatin was prepared from clean nuclei as reported before[24]. The incorporation of ^{3}H-glucosamine into various molecular weight classes of proteins from the three nuclear preparations was determined by electrophoretic fractionation according to molecular weight in sodium dodecyl sulphate (SDS)–polyacrylamide gels.

Significant levels of incorporation of ^{3}H-glucosamine into chromatin-associated non-histone polypeptides which migrate

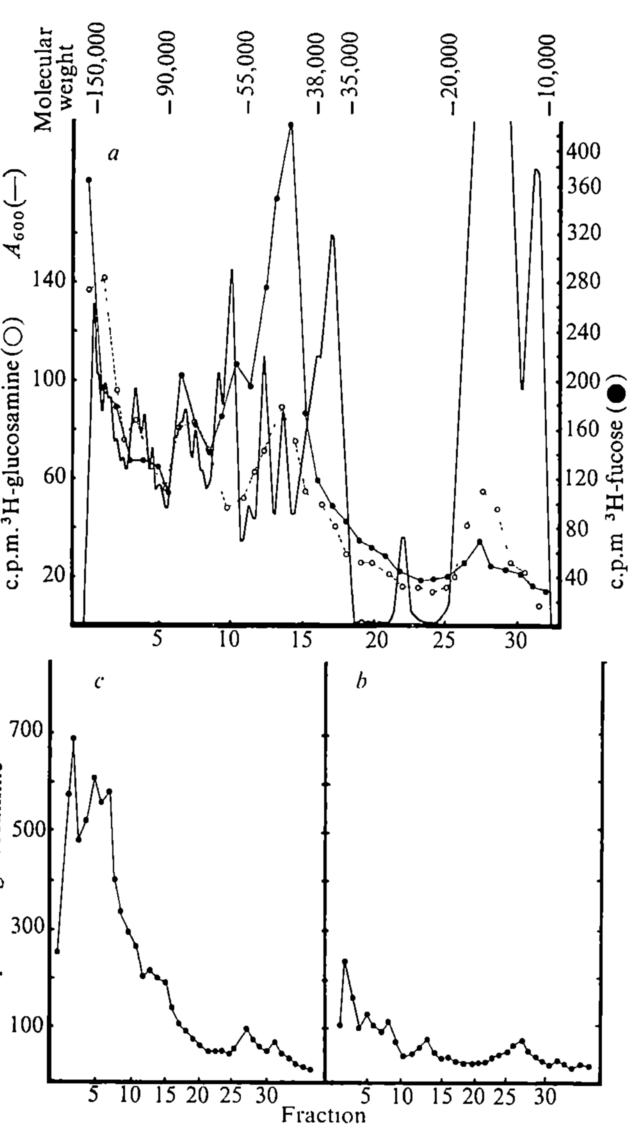

Fig. 1 SDS–polyacrylamide gel electrophoretic fractionation of proteins from chromatin (*a*), clean nuclei (*b*) and crude nuclei (*c*). Nuclear material was dissociated in 1% SDS, 1% β-mercaptoethanol and 0.01 M sodium phosphate, *p*H 7.0, and dialysed against 0.1% SDS, 0.1% β-mercaptoethanol and 0.01M sodium phosphate, *p*H 7.0. Electrophoresis was carried out as described before in 0.6×7.5 cm, 7.5% polyacrylamide gels with 0.6×2 cm, 2.3% stacking gel[35].

in the 150,000–90,000 (fractions 1–6), 90,000–55,000 (fractions 7–10), and 55,000–38,000 (fractions 11–16) molecular weight regions of SDS–polyacrylamide gels are evident (Fig. 1*a*). Incorporation of ^{3}H-glucosamine is also observed in the 10,000–15,000 molecular weight region of the gel (fractions 26–30), where histone polypeptides migrate. But since tryptophan-containing non-histone chromosomal proteins also migrate in the 10,000–15,000 molecular weight region of these gels, our data do not establish unambiguously whether these ^{3}H-glucosamine containing polypeptides are histones or low molecular weight non-histone chromosomal proteins.

When chromosomal proteins from cells labelled with ^{3}H-glucosamine were subjected to acid hydrolysis[25] followed by paper chromatography[26], the ^{3}H was found primarily in the glucosamine and galactosamine, eliminating the possibility that the ^{3}H-glucosamine was metabolised to components other than amino sugars. Further evidence that glycoproteins are genome components is provided by the incorporation of ^{3}H-L-fucose into chromosomal polypeptides (Fig. 1*a*). Although ^{3}H-fucose is incorporated primarily into the same molecular weight classes of chromosomal proteins as those which incorporate ^{3}H-glucosamine, there are pronounced differences in the relative extents to which the two labelled precursors are incorporated. This suggests variations of the carbohydrate components of specific molecular weight classes of chromosomal proteins. The ^{3}H-glucosamine and ^{3}H-fucose containing materials which migrate in a heterodisperse manner in SDS–polyacrylamide gels are degraded in the presence of Pronase, confirming that the carbohydrate groups are associated with proteins and not with other types of macromolecules.

Several approaches were used to establish that the glycoproteins associated with chromatin are *bona fide* genome components rather than membrane material which becomes associated with chromatin during the isolation process. When trypsinates (material removed from cells by treatment with 0.25% trypsin for 15 min in Hanks' balanced salt solution) from cells labelled with ^{3}H-glucosamine were added to unlabelled HeLa cells before chromatin isolation, no significant levels of radioactivity were recovered with the chromatin preparations. Similarly, when isolated plasma membranes[27] prepared from cells labelled with ^{3}H-glucosamine were added to unlabelled HeLa cells before chromatin isolation, there were no significant levels of radioactivity in the chromatin. Together these results indicate that the labelled macromolecules associated with our chromatin preparations are not contaminating cell surface material. Figure 1*b* and *c* shows the SDS–polyacrylamide gel electrophoretic radioactivity profiles of ^{3}H-glucosamine-labelled polypeptides of crude nuclei and clean nuclei. While comparison of the electrophoretic profiles of these nuclear preparations with that of chromatin suggests a similar distribution of glycoproteins in the low molecular weight regions of the gels (10,000–35,000), significant quantitative, and perhaps qualitative, variations are evident in the higher molecular weight regions.

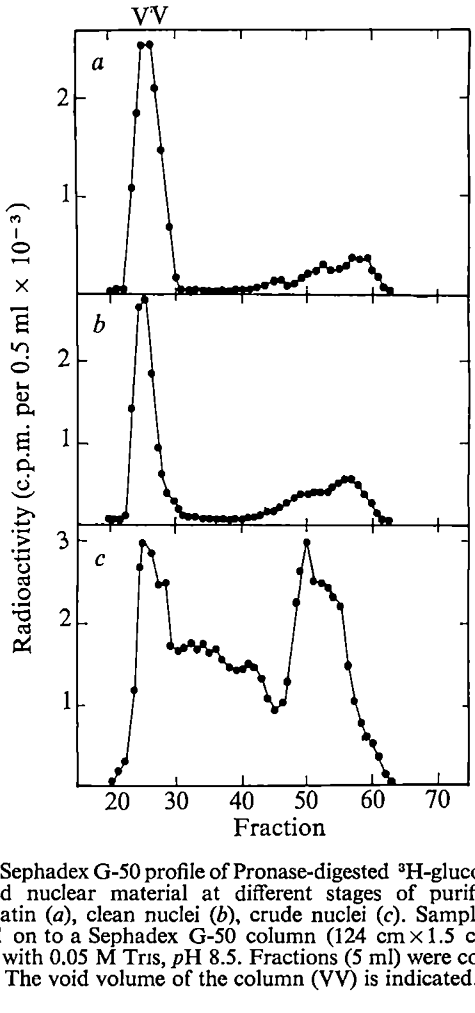

Fig. 2 Sephadex G-50 profile of Pronase-digested ^{3}H-glucosamine labelled nuclear material at different stages of purification: chromatin (*a*), clean nuclei (*b*), crude nuclei (*c*). Samples were loaded on to a Sephadex G-50 column (124 cm×1.5 cm) and eluted with 0.05 M Tris, *p*H 8.5. Fractions (5 ml) were collected. The void volume of the column (VV) is indicated.

Although these findings by no means conclusively eliminate the presence of nuclear membrane material in our chromatin preparations, the pronounced differences in the glycoproteins of nuclei and chromatin preclude the possibility that the glycoproteins associated with chromatin are random contaminants from the nuclear membrane. It should be noted that when chromatin is purified by centrifugation through 1.7 M sucrose, which should eliminate membrane material, more than 90% of the glycoproteins present in the chromatin preparation remain associated with the purified chromatin pellet. The polyacrylamide gel electrophoretic profiles of these chromatin preparations before and after sucrose sedimentation are indistinguishable. When ^{3}H-thymidine-labelled chromatin is subjected to this purification procedure, a similar distribution of DNA between the pellet and supernatant is observed. If selected membrane-derived glycoproteins are isolated with chromatin, these may represent specific sites of interaction between the genome and the nuclear membrane. The possibility should also be considered that carbohydrate-containing nuclear material—glycoproteins and glycosaminoglycans (discussed below)—constitutes components of the annulate lamellae [28] or nuclear pore complex[29–31].

Further characterisation of the carbohydrate-containing nuclear material labelled with ^{3}H-glucosamine was carried out as follows. Crude nuclei, clean nuclei, and chromatin were subjected to Pronase digestion to hydrolyse polypeptides, and Pronase-resistant material was analysed by Sephadex G-50 chromatography (Fig. 2). In each run the sample used originated from a comparable number of labelled cells. Clearly the material of high molecular weight, eluting at the void volume of the column, accompanied the chromatin throughout the purification procedure. Furthermore, there was little qualitative or quantitative difference between the clean nuclei and final chromatin preparations in terms of the distribution of radioactivity among different size fractions of glycopeptides. The original crude preparation of nuclei, however, contained considerably more materials which were partially or wholly included by the gel. The high molecular weight nature of the ^{3}H-glucosamine-labelled material from HeLa chromatin, which was excluded from the Sephadex G-50 column, was confirmed by chromatography on a BioGel P-100 column when it again eluted with the excluded volume. This carbohydrate-containing material was analysed further by ion exchange chromatography on a DEAE-cellulose column using a linear gradient of ammonium acetate as eluant[32,33]. Most of the radioactivity eluted at salt concentrations (1.0–1.5 M) which characteristically elute chondroitin sulphates. Hyaluronic acid, a non-sulphated glycosaminoglycan, which elutes at approximately 0.6 M, was clearly not present. Since the high molecular weight ^{3}H-labelled material was hydrolysed both by hyaluronidase and by chondroitinase, and heparan sulphates are not, it is reasonable to conclude that the labelled material is a glycosaminoglycan in the chondroitin class. This conclusion is reinforced because acid hydrolysis of the labelled material releases a radioactive monosaccharide component with the chromatographic mobility of galactosamine. No radioactivity from this high molecular weight material was found in glucosamine, the amino sugar component of hyaluronic acid and heparan sulphates. Furthermore, the polymer was not degraded by nitrous acid[34].

Our results suggest that at least two classes of carbohydrate-containing materials are associated with the isolated genome—chromatin—of HeLa S_3 cells. Glycoproteins with molecular weights ranging between 10,000 and 190,000, as well as glycosaminoglycans of high molecular weight have been identified. The latter do not seem to migrate into SDS-electrophoretic gels in the conditions we used. Characterisation of glycosaminoglycans as components of the eukaryotic nucleus is consistent with a recent report by Bhavanandan and Davidson[34]. Although the significance of our findings remains to be resolved, a role for glycoproteins and mucopolysaccharides in the structure or transcriptional and replicative functions of the genome can be considered.

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Different degrees of interspecies homology in immunoglobulin λ chain constant domain correlated with three-dimensional structure

IT is generally accepted that evolution of proteins is a consequence of duplications and point mutations occurring in genes coding for them. Palaeogenetics of Pauling and Zuckerkandl[1] as well as protein phylogenetics introduced by Fitch and Margoliash[2] (and subsequently modified by other workers) assume that comparative analyses of amino acid sequence data in terms of mutational distances (be it minimal mutational distances, corrected mutational distances or accepted point mutations per 100 amino acids), inferred to exist among genes coding for them, yield a realistic image of evolution; thus, genealogical trees of proteins (that is, dendrograms of mutational distances) can be constructed which relate to main evolutionary events thought to occur in the past.

Alternatively, Epstein[3,4] suggested that the requirement to conserve a correct tertiary structure is the main governing force of protein evolution, the point mutations being mere driving forces of the evolution (this is because "biological function is more a correlate of macromolecular geometry than

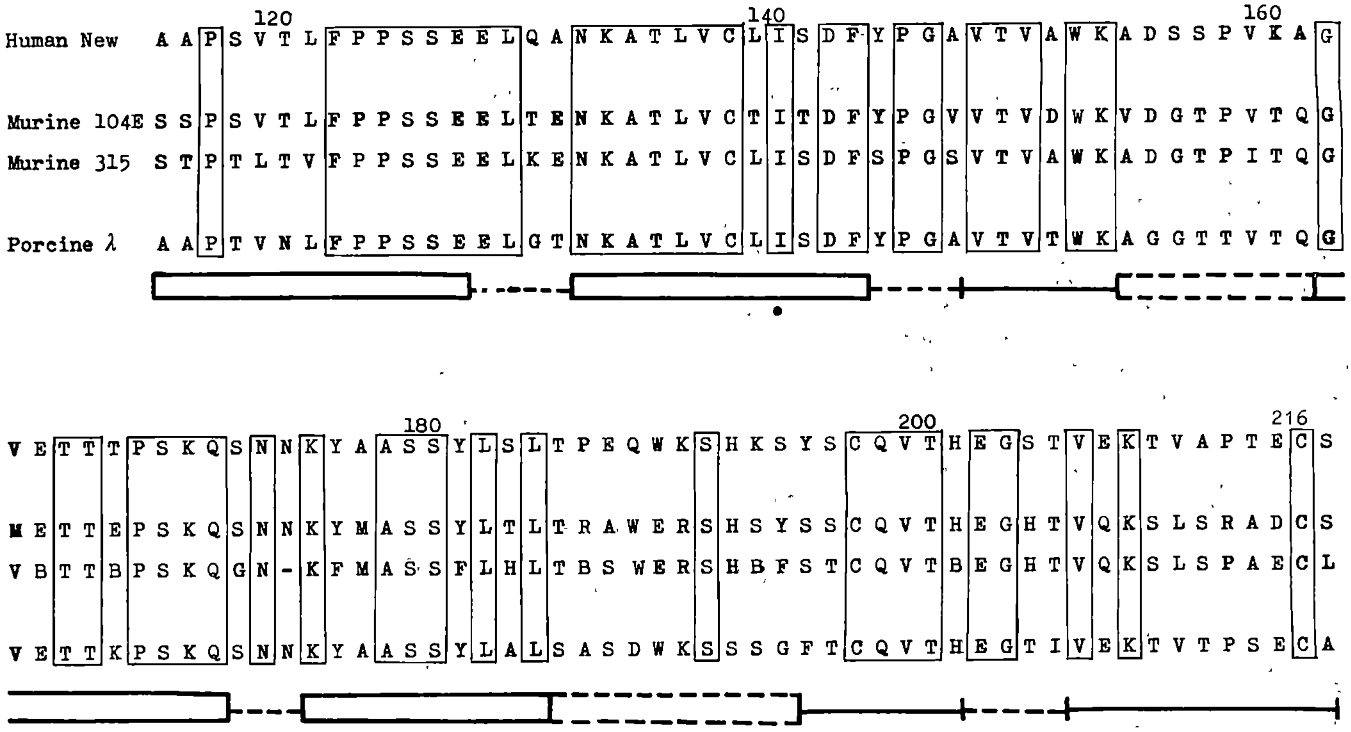
120
140
160
Human New A A P S V T L F P P S S E E L Q A N K A T L V C L I S D F Y P G A V T V A W K A D S S P V K A G
Murine 104E S S P S V T L F P P S S E E L T E N K A T L V C T I T D F Y P G V V T V D W K V D G T P V T Q G
Murine 315 S T P T L T V F P P S S E E L K E N K A T L V C L I S D F S P G S V T V A W K A D G T P I T Q G
Porcine λ A A P T V N L F P P S S E E L G T N K A T L V C L I S D F Y P G A V T V T W K A G G T T V T Q G
180
200
216
V E T T T P S K Q S N N K Y A A S S Y L S L T P E Q W K S H K S Y S C Q V T H E G S T V E K T V A P T E C S
M E T T E P S K Q S N N K Y M A S S Y L T L T R A W E R S H S Y S S C Q V T H E G H T V Q K S L S R A D C S
V B T T B P S K Q G N - K F M A S S F L H L T B S W E R S H B F S T C Q V T B E G H T V Q K S L S P A E C L
V E T T K P S K Q S N N K Y A A S S Y L A L S A S D W K S S S G F T C Q V T H E G T I V E K T V T P S E C A

Fig. 1 Amino acid sequences of constant regions of immunoglobulin λ chains. Amino acid sequences are given in a one-letter notation for amino acid sequences[22]. Positions are numbered from the N-terminus. Sources of data: porcine normal λ chains[12]; human myeloma New[13]; murine myeloma 104E (ref. 14), murine myeloma 315 (ref. 15). The residues common to all sequences compared are indicated by a frame. Code for elements of the tertiary structure[13,16]: ═══, four-segment layer of the antiparallel β pleated sheet; ———, three-segment layer of the antiparallel β pleated sheet; — — — —, hairpin bends; = = = = =, loops at the surface of the domain.

of chemical detail"[5]); according to this view, analysis of amino acid sequence data in terms of mutational distances are irrelevant[6]. Rather, the sequence data should be analysed in terms of (1) regions of primary structure which correspond to main elements of the three-dimensional structure (that is, α helices, β pleated sheets, β bends, and so on) and (2) constraints imposed by correct folding on free amino acid interchangeability in these regions, in relation to chemical homologies existing among side chains of amino acids under consideration[7].

Most of the protein dendrograms fit palaeontological data well, seemingly supporting concepts of palaeogenetics and protein phylogenetics. On the other hand, results of crystallographic[8] and model-building[9] studies tend to agree with Epstein's view. Also, statistical analyses of codon variability carried out by Fitch and Markowitz[10] and by Holmquist[11] do suggest that, in the course of evolution, (three-dimensional) constraints influence amino acid interchangeability in proteins in a decisive way. Obviously, further experimental data may provide better understanding of the problem.

We have determined the primary structure of the constant region (that is, a C-proximal half) of pig immunoglobulin λ chains[12]. This structure is particularly suitable for evolutionary studies: amino acid sequences of constant regions of human[13] and murine[14,15] λ chains are known, together with the three-dimensional structure of two human λ chains (New [13] and Mcg[16]). The constant regions of these latter two chains fold independently from their variable regions (that is, N-proximal halves of the chain), representing an autonomous protein domain; the overall pattern as well as the details of the folding are almost identical in both the constant domains. The same is true for the constant domain of a mouse myeloma κ chain[17] and it seems reasonable to assume that the same folding pattern exists in other mammalian species.

For comparison, we considered separately those segments of primary structure of λ chain constant domain which correspond to elements constituting the three-dimensional structure of the domain, namely, two layers of antiparallel β pleated sheets (the first one composed of four and the second of three polypeptide chain segments), four short hairpin bends and two loops at the surface of the domain (Fig. 1). The segments homologous in λ chains from different organisms were compared according to two alternative procedures. First, the number of identical amino acid residues was evaluated (Table 1). Second, from the number of amino acid replacements observed in one segment with

Table 1 Comparison of polypeptide chain segments from the constant region of immunoglobulin λ chains

	4 Segments of β-pleated sheet layer		3 Segments of β-pleated sheet layer		4 Hairpin bends		2 Surface loops		Whole domain	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
Mouse 104E–Mouse 315	75	10	75	8	67	18	67	13	71	11
Pig–Mouse 104E	82	9	54	15	67	18	39	31	67	16
Pig–Mouse 315	80	10	62	14	47	29	39	30	64	17
Pig–Man	95	3	75	8	73	11	33	30	75	10
Man–Mouse 104E	87	8	62	12	73	13	33	30	69	14
Man–Mouse 315	82	10	62	11	53	22	33	31	63	16
Range	75–95	3–10	54–75	8–15	47–73	11–29	33–67	13–30	63–75	11–17
% Identical amino acid residues in all sequences compared	67		50		47		6		51	

A, Percentage identical amino acid residues in this category of segments; *B*, minimal mutational distance per 100 nucleotides

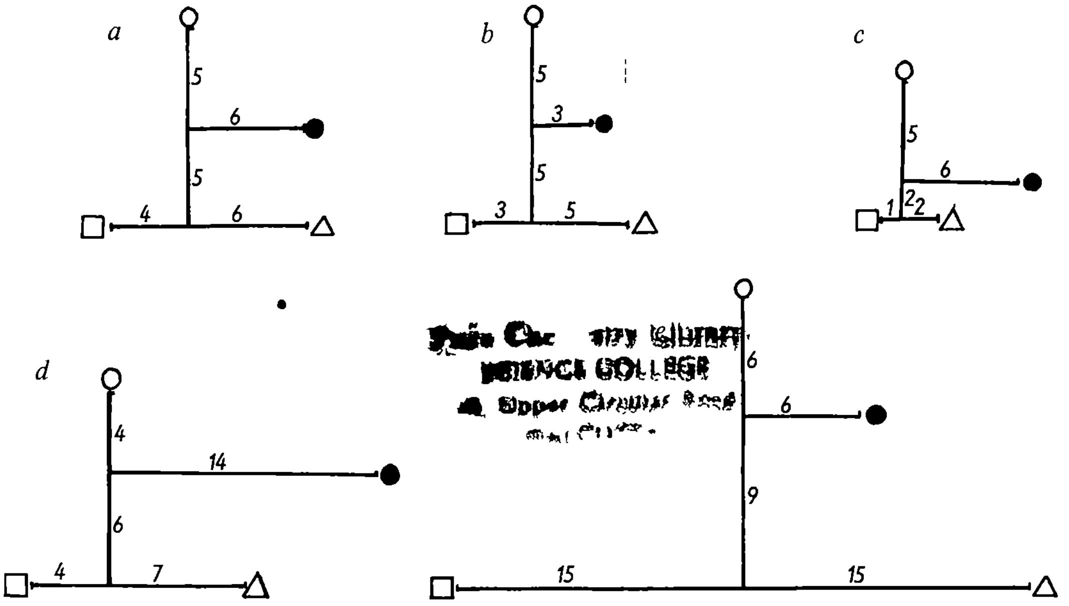

Fig. 2 Dendrograms constructed from mutational distances of segments of λ chain constant domain homologous in different organisms. *a*, Whole domain; *b*, three-segment layer of the β pleated sheet; *c*, four-segment layer of the β pleated sheet; *d*, hairpin bends; *e*, surface loops. Numbers represent mutational distances per 100 nucleotides. □, Man; △, pig; ○, mouse 104E; ●, mouse 315.

respect to the other one, minimal mutational distance[2] of that pair was calculated (Table 1). The mutational distances were used to construct dendrograms (Fig. 2).

By the first procedure, segments involved in non-covalent interactions of the domain core (four-segment layer of the β-pleated sheet and, to a lesser extent, three-segment layer of the β-pleated sheet as well as the hairpin loops) were found to be highly conservative in terms of amino acid substitutions, whereas segments which are exposed to the surface of the domain and only marginally participate in the folding architecture (surface loops) are much less conservative (Table 1).

The second procedure shows that segments corresponding to different elements of the three-dimensional structure accumulate mutations at a different rate determined by their particular folding constraints ("conservation laws"), the constraints causing the accepted amino acid substitutions to be non-random events[18]. Accordingly, the surface loops, being subject to only faint constraints, display a dendrogram which approaches the phylogenetic tree of the species derived from palaeontological data. The same is true for the dendrogram of the whole domain. In contrast, the four-segment layer of the β-pleated sheet, being a subject to very strong constraints, displays a dendrogram with quite unnatural and distorted "evolutionary distances". For example, the (intraspecies) mutational distance between mouse λ chain subtypes would be, according to this dendrogram, almost six times longer than the (interspecies) distance between pig and man.

We thus conclude that the dendrograms constructed from mutational distances of short segments of primary structure (a procedure widely used among immunochemists) often lead to erroneous results. Figure 2 shows that only large regions of primary structure (such as the whole domain) or segments under exceptionally low folding constraints (such as surface loops) can serve for calculation of mutational distances which reasonably approximate the course of evolution. Our analysis also shows that protein genealogical trees (commonly thought to represent reconstructions of a time course of supposed evolutionary events) arise as consequences of "amino acid selection rules", that is, constraints imposed by folding requirements on free amino acid interchangeability in proteins[19].

A large body of primary structures of immunoglobulin polypeptide chains has accumulated but theoretical studies based on these structures have mostly neglected the unique three-dimensional aspects of the "immunoglobulin fold", comparing the structure only from the point of view of underlying mutational distances. Taking into account folding peculiarities of immunoglobulins, a plausible explanation[19–21] might be provided for some of the paradoxical features of antibody molecules.

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Human fibrinogen possesses binding site for staphylococci on Aα and Bβ polypeptide chains

THE fibrinogen molecule, which comprises 5% of plasma proteins, is involved in enzymatic reactions responsible for formation and dissolution of the fibrin clot[1]. Thereby, fibrinogen is being transformed by thrombin into fibrin, which is cross linked by glutaminyltransamidase (factor XIII), and ultimately degraded by plasmin. The Aα, Bβ and γ polypeptide chains (nomenclature according to the recommendations of the Committee on Nomenclature of the International Society on Thrombosis and Haemostasis, 1971) of fibrinogen involved in these reactions and the sites of enzymatic attack thereon are defined[2–8]. In addition to

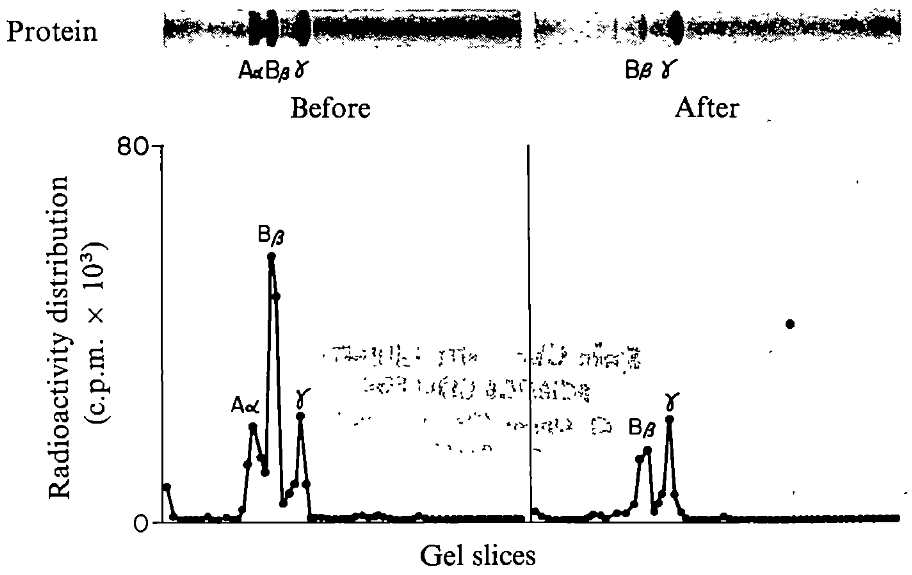


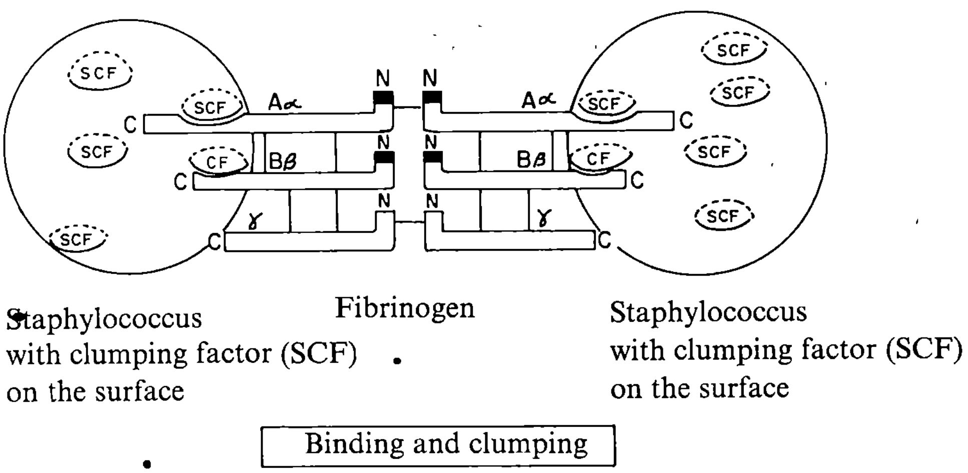
Fig. 1 Interaction of staphylococci with polypeptide chains of reduced and alkylated ^{125}I-human fibrinogen. Before interaction with staphylococci, three major bands representing Aα, Bβ and γ chains and corresponding three radioactivity peaks could be distinguished. After interaction, the Aα chain and part of Bβ chain disappeared indicating their binding to staphylococci. γ Chain remained in solution. SDS–polyacrylamide gel electrophoresis was performed according to the procedure of Weber and Osborn[16]. Gels were stained with Coomassie blue and sliced for radioactivity measurement.

being involved in the enzymatic reactions responsible for formation and dissolution of the fibrin clot, fibrinogen exhibits the ability to interact in a non-enzymatic reaction with pathogenic staphylococci to cause their clumping[9]. Clumping of staphylococci is used to detect fibrinogen and its derivatives in blood and body fluids[10,11]. This reaction can detect as little as 0.03 μg human fibrinogen or its derivatives. The mechanism through which human fibrinogen interacts with staphylococci is however, unexplained and the localisation of the binding site(s) for staphylococci remains unknown. We therefore studied localisation of the binding site for staphylococci on human fibrinogen. We now report that the binding site for staphylococci is located on Aα and Bβ chains of human fibrinogen.

We used human fibrinogen (Kabi) labelled with ^{125}I according to the method of McFarlane[12] and reduced and alkylated to have separate polypeptide chains[13]. A mixture of polypeptide chains of fibrinogen was incubated with staphylococci in the presence of 3 M urea which does not inhibit the clumping reaction[14,15]. Staphylococci with bound subunits of fibrinogen were removed by centrifugation and the supernatant was analysed for residual, unbound material. As it is shown in Fig. 1, before interaction with staphylococci we could distinguish in SDS–polyacrylamide gel three major bands representing Aα, Bβ and γ chains of human fibrinogen. Accordingly, the radioactivity pattern showed three peaks in the sliced gel

After the interaction between reduced and alkylated fibrinogen and staphylococci, Aα and part of Bβ chains disappeared as shown by protein staining and radioactivity peaks in the sliced gel (Fig. 1). In contrast, the γ chain remained in solution, indicating lack of its binding to staphylococci To gain a more precise insight into the extent of binding of individual chains of fibrinogen with staphylococci, we measured the amount of radioactivity in the SDS–polyacrylamide gel before and after interaction with staphylococci and computed the percentage radioactivity bound to staphylococci. The results of three independently conducted experiments (mean ±s.d.) were as follows. When we took ^{125}I-human fibrinogen which was not reduced, 91.7±10.2% of radioactivity was bound to staphylococci. After reduction and alkylation of ^{125}I fibrinogen 95.0±2.0% of Aα chain and 65.3±11.9% of Bβ chain interacted with staphylococci. In contrast, only 23±9.0% of radioactivity associated with γ chain has disappeared from the solution after interaction with staphylococci. Thus, these experiments indicate the high affinity of Aα chain of human fibrinogen for staphylococci and the lower affinity of Bβ chain γ Chain possesses very low, if any, affinity towards staphylococci.

The fact that Aα and Bβ chains of human fibrinogen possess the staphylococci-binding site raises the next question Do individual chains of human fibrinogen possess one or more binding sites for staphylococci? To answer this question, we compared the binding and clumping of staphylococci by non-reduced and reduced fibrinogen. Whereas non-reduced fibrinogen demonstrated excellent correlation between binding (91.7%) and clumping (titre 1024), reduced fibrinogen showed only significant binding (60%). The clumping activity of reduced fibrinogen was greatly diminished (titre 0–2) This suggests that individual chains separated by reduction of fibrinogen cannot link two


SCF
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Aα
Aα
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Bβ
γ
γ
CF
SCF
SCF
SCF
SCF
C
C
C
Staphylococcus with clumping factor (SCF) on the surface
Fibrinogen
Staphylococcus with clumping factor (SCF) on the surface
Binding and clumping

Fig. 2 Diagrammatic presentation of interaction between human fibrinogen and staphylococci. Binding site for staphylococcal clumping factor (SCF) is localised on Aα and Bβ chains between the N-terminal disulphide knot and the C-terminal end of the chains. At least two polypeptide chains with one binding site each need to be bound together by disulphide bridges to join two staphylococci. The dimeric structure of native fibrinogen molecule thus permits not only binding but also clumping of staphylococci.

or more staphylococci together to cause their clumping. Aα and Bβ chains must be bound by disulphide bridges to clump staphylococci. Thus we can conclude that individual Aα and Bβ chains possess only one binding site, since the clumping reaction, like most agglutination reactions, requires bivalent molecules which in this instance are represented by the dimeric structure of intact fibrinogen.

The following lines of evidence indicate that the binding site for staphylococci is located on the C-terminal portion of Aα and Bβ chains of human fibrinogen. First, the binding sites were gradually destroyed during progressive degradation of the C-terminal portions of Aα and Bβ chains with plasmin. Second, isolated fragment E (ref. 7) labelled with ^{125}I and isolated N-terminal disulphide knot obtained after CNBr-treatment of fibrinogen[17] and labelled with ^{125}I did not bind or clump staphylococci (J.H , D.K.H., S.T. and A. Z. Budzynski, unpublished). Since both the N-terminal disulphide knot and fragment E represent that segment of the fibrinogen molecule which is devoid of the C-terminal portion of polypeptide chains, the lack of binding and clumping of staphylococci suggests that the binding site is located on the missing C-terminal portion of Aα and Bβ chains.

These data represent the initial demonstration that human fibrinogen possesses a binding site for staphylococci on Aα and Bβ chains. The binding site is located between the N-terminal disulphide knot and the C-terminal end of Aα and Bβ chains. The dimeric structure of the fibrinogen molecule facilitates not only binding but also clumping as shown in Fig. 2. When the fibrinogen molecule is reduced, individual Aα and Bβ chains interact with staphylococci but there is no clumping of staphylococci. This is because a least two chains with one binding site each need to be bound together by disulphide bridges in order to be able to link two staphylococci.

Demonstration of the binding site for staphylococci on Aα and Bβ chains as described herein explains the mechanism of the clumping of staphylococci by human fibrinogen and its derivatives. It also offers a new approach to study the structure and the function of subunits of fibrinogen molecule. By using staphylococci it is possible to selectively absorb individual subunits of human fibrinogen. Similarly, our findings that the binding site for staphylococci is located beyond that region of the fibrinogen molecule from which fibrinopeptides are released or which participates in the polymerisation step, provides the basis for use of staphylococci to measure abnormal variants of fibrinogen. Furthermore, fibrinogen has been reported to have a role in intravascular immunological disorders[18] which can be triggered by some microbial agents. The interaction between human fibrinogen and staphylococci may serve as an example of non-enzymatic reaction involving fibrinogen and possibly leading to its deposition around microbial antigens as postulated in some immune complex diseases. This function of clottable protein to interact with foreign particles in the environment of blood is phylogenetically an old phenomenon occurring in primitive species possessing clottable protein confined within haemocytes[19]. Preservation of the binding site for staphylococci on several mammalian fibrinogens studied[20] indicates that this property has been quite constant during mammalian evolution.

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Induced changes in gene conversion spectra

IN crosses, segregation at a heterozygous site may produce a low frequency of octads with spore ratios of 6+ : 2*mutant* or 2+ : 6*m* (meiotic segregation), or 5+ : 3*m* or 3+ : 5*m* (postmeiotic segregation), instead of the usual 4+ : 4*m* ratio. These irregular (aberrant) segregations are usually attributed to gene conversion involving hybrid DNA formation at the heterozygous site, with or without enzymatic correction of the resulting base mispairs. If the frequency and direction (to + and to *m*) of correction depend on the chemical nature of the mispair, then the chemical origin of the mutation involved in the mispair could affect its conversion spectrum, providing evidence in favour of hybrid DNA recombination models.

Leblon[1] showed a clear relationship between the molecular origin of induced mutations and their conversion spectra. He defined conversion spectra by two criteria—the frequency of postmeiotic segregation, and the relative frequencies of conversion to the mutant allele or to wild type. Four main kinds of spectrum were recognised and, by the use of specific mutagens and reversion tests, were identified with different kinds of molecular mutation. The studies reported here show, however, that the conversion spectrum for a single mutation can vary with genetic and environmental factors, thus a particular mutation giving one type of spectrum under one set of conditions may give a different type of spectrum under other conditions, although the mutation itself is unaltered.

The spectrum types and their associated kinds of mutation given by Leblon[1] were: type A, deletion frame-shifts, rare postmeiotic segregation and excess of conversion to wild type, type B, addition frame-shifts, rare postmeiotic segregation and excess of conversion to mutant; type C, base substitutions, postmeiotic segregation not rare, excess of conversion to wild type; type D, base substitutions, postmeiotic segregation not rare, excess of conversion to mutant.

We have used Pasadena strains of *Ascobolus immersus*, with methods, controls for repeatability, phenocopies, new mutations and false spore-clusters as described before[2–4]. *w*-10 and *w*-78 are very closely linked, possibly allelic, spontaneous white ascospore mutations. The two original mutants gave 2–4% conversion in crosses to wild type, while certain strains isolated from 2+ : 6*w* octads in such crosses gave much higher conversion frequencies, 8–20% (refs 2 and 4). The reisolated mutants, referred to as HCF (high conversion frequency) strains, are isoallelic

Table 1 Variation in gene conversion parameters with: (1) genetic factors; (2) temperature, in + × *w* crosses of *Ascobolus immersus*

Cross	Temp. (° C)	Total octads	(*a*) $6+:2w$ + $2+:6w$ %	(*b*) $5+:3w$ + $3+:5w$ %	(*c*) $\frac{6+:2w + 2+:6w}{5+:3w + 3+:5w}$	(*d*) $\frac{6+:2w}{2+:6w}$	(*e*) $\frac{5+:3w}{3+:5w}$
(i)							
LCF *w*-78 × P5	17.5	18,406	3.77	0.47	8.0	0.40	1.04
HCF *w*-78 × P5	17.5	2,832	9.00	1.13	8.0	1.60	1.69
LCF *w*-78 × 1WT3	17.5	7,637	2.13	0.24	8.9	1.01	1.40
HCF *w*-78 × 1WT3	17.5	2,958	8.01	0.65	12.3	2.48	0.59
LCF *w*-10 × P5	17.5	18,034	3.25	0.31	10 5	0.64	0.72
HCF *w*-10 × P5	17.5	55,418	13.48	0.29	46.5	2.75	1.42
LCF *w*-10 × 81	17.5	5,570	4.45	0.71	6.3	1.21	0.25
HCF *w*-10 × 81	17.5	12,640	18.07	0.11	164.3	3.16	2.67
(ii)							
LCF *w*-78 × P5	10.0	23,503	0.99	0.40	2.5	0.35	3.70
	17.5	18,406	3.77	0.47	8.0	0.40	1.04
	22.5	5,418	2.69	0.29	9.1	2.40	0.61
HCF *w*-78 × P5	10.0	9,845	4.84	0.78	6.2	1.45	1.48
	17.5	2,832	9.00	1.13	8.0	1.60	1.69
	22.5	6,733	10.47	0.99	10.5	4 55	2.53
LCF *w*-10 × P5	10.0	4,853	1.23	0.16	6.7	0.13	7.00
	17.5	18,034	3.25	0.31	10.5	0.64	0.75
	22.5	11,328	2.22	0 21	10.5	3.05	0.50
HCF *w*-10 × P5	10.0	15,134	5.22	0.15	34.4	2.46	1.09
	17.5	53,230	13.55	0.31	44.8	2.76	1.40
	22.5	1,389	14.62	0.79	18.5	4.21	0.83

a, Total observed meiotic segregation frequency; *b*, total observed postmeiotic segregation frequency; *c*, ratio of observed meiotic and postmeiotic segregation; *d* and *e*, ratio of correction to wild type and to mutant in octads with (*d*) meiotic and (*e*) postmeiotic segregation.

with the original low converting (LCF) strains but differ from them in a very closely linked factor controlling their conversion frequency.

Data in Table 1i, comparing LCF and HCF crosses (of *w*-10 and *w*-78 to wild-type P5 and derived wild types 1WT3 or 81), show that gene conversion parameters varied with the genetic factor, which had major effects on conversion spectra as well as on conversion frequencies Thus in crosses to P5, conversion was more often to mutant than to wild type in the LCF crosses, but the reverse was true in HCF crosses. The factor affected the absolute frequencies of observed meiotic and postmeiotic segregation, and also the frequencies of meiotic segregation relative to postmeiotic segregation. For example, in *w*-10 crosses with 81, meiotic segregation was six times more frequent than postmeiotic segregation in the LCF cross but was 164 times more frequent in the HCF cross.

Table 1ii shows that temperature also had major effects on conversion spectrum criteria. Thus for 6 : 2 and 2 : 6 octads in LCF crosses *w*-78×P5 and *w*-10×P5, conversion was much more often to mutant than to wild type at 10 °C and 17 5 °C but the reverse occurred at 22.5 °C. A similar reversal, with an opposite trend, is shown for the corresponding 5 : 3 and 3 : 5 octads. The absolute frequency of asci with postmeiotic segregation showed various changes with temperature; the relative frequencies of meiotic and postmeiotic segregation also changed; for example, for LCF *w*-78×P5, from 2.46 at 10 °C to 9.13 at 22.5 °C. Many of these changes are significant at $P=0.01$ or 0.05.

These effects of genetic and environmental factors on conversion spectra show that a given mutation may have one type of conversion spectrum (A, B, C or D, as defined by Leblon[1]) in one set of circumstances but another kind in different conditions even though the mutation itself is unchanged. Thus LCF strains of *w*-78 and *w*-10 had spectrum type D at 10 °C and 17.5 °C, but type C at 22 5 °C. *w*-78 and *w*-10 LCF strains had spectrum type D at 10 °C and 17.5 °C but the corresponding HCF strains, carrying the same mutations, were of type C at those temperatures. The range 17.5 °C–22.5 °C is regarded as normal for making crosses of these Pasadena strains of *Ascobolus*, yet even within this narrow range the original mutants (LCF strains of *w*-78 and *w*-10) had different types of conversion spectrum, D or C.

The spectrum criterion of relative frequencies of conversion to wild type and to mutant can thus be changed for a particular mutant (Table 1, columns *d* and *e*). The other criterion, the frequency of postmeiotic segregation, was less clearly defined by Leblon[1], being used on its own and also as a comparison of meiotic and postmeiotic segregation frequencies. Table 1, column *c*, shows that large changes in relative frequencies of meiotic and postmeiotic segregation can occur for a mutation.

Work with one standard set of environmental and genotypic conditions may therefore not reveal the range of conversion spectra which a single mutation can show. Because mutations may differ in their conversion responses to genetic and environmental factors[4,5], different mutants might have different conversion spectrum types in any single set of conditions, even if those mutations are of the same chemical type. In Leblon's data[1] (his paper I, Tables 3–11) the differences between conversion spectrum types were clear for 72 out of 92 mutants, and in very distinctive cases qualitative changes of spectrum type by environmental or genotypic differences are presumably less likely than with the mutants used in our experiments.

Our results show that types of conversion spectrum for single mutations can vary with genetic and environmental factors. Deductions from conversion spectra alone about the molecular nature of any mutation should therefore be regarded as very tentative unless backed by evidence from mutation and reversion studies.

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reviews

Portrait of the philosopher

Asa Briggs

This huge biography* has many of the virtues and some of the defects of the huge nineteenth-century biographies which Lytton Strachey made fun of while at the same time ransacking them. It begins somewhat pretentiously and ends somewhat tendentiously, and the tone, not to speak of the style, is uneven throughout. There are far too many examples of "was to" and "would be". No Victorian biographer, of course, would have dealt as explicitly or at such length with Russell's sexual adventures as does Ronald Clark, but the treatment of these is not dissimilar from the way a Victorian would have dealt with them had he tried. There is a vast amount of unnecessary detail, and a fair amount of superfluous and repetitive commentary. Yet Clark is doubtless right to refuse to deal with Russell without revealing "the deep emotional complexities" of his personality. Russell himself—at least on one occasion—asked for a biographer who would not treat him as "a solemn stained glass saint existing only for purposes of edification". Like any Bloomsbury character—and such characters dominate many of the middle pages of this volume—he insisted, a little too indulgently, that a biographer would have to recognise above all that "I existed from my own centre."

It was a way of putting things which left a great deal out. It left out, for example, his Whig inheritance which could be identified more specifically than Clark does, and a corresponding set of Whig attitudes which he never entirely shook off. He used popular feelings when he chose to do so, but he never entirely shared them, and he knew instinctively, when he chose to do so, how to appeal to people with power. He was always interested in the relationship between freedom and power, and he never doubted that people should be listening to him on the subject. "I can understand your envying me," he once wrote to Nehru, "because a private person is not obliged to bear in mind the very complex considerations which beset a head of state."

Bertrand Russell as a freshman at Trinty College, Cambridge

With Russell's Whiggery went a strong sense of the need for an intellectual aristocracy. "Surely one Darwin is more important than 30 million working men and women." The controversial Ralph Schoenman in a passage of admirable clarity, the kind of clarity which Russell himself often achieved and always appreciated, wrote that there were "serious intellectual doubts which inhibited him from engaging wholeheartedly in mass movements with revolutionary aims. He felt that cultural excellence and unique achievement were the product of favoured circumstance."

Russell's own unique achievement is dealt with fairly in this biography. He turned to mathematics as a boy with delight, quickly choosing the most abstract parts" which he understood best. He needed to detach himself in order to pursue mathematical studies. "I like mathematics," he wrote, "because it is not human and has nothing particular to do with this planet or with the whole accidental universe—because, like Spinoza's God, it won't love us in return." Although he wrote and published *German Social Democracy* before *A Critical Exposition of the Philosophy of Leibniz*, he was stimulated and challenged more by Frege than by Bebel. The intellectual collaboration with Whitehead, which culminated in *Principia Mathematica*, is dealt with fully by Clark: so, too, less fully, is Russell's relationship with Wittgenstein. There is a great deal of relevant unpublished source material in the Bertrand Russell Archives at McMaster University, which Clark has carefully studied, and he has no difficulty in showing in what areas Russell pioneered and on what issues he himself changed his mind. All the references are useful, even when they are not conclusive. There is far more to be written, however, possibly in essays, about Russell's influence on other philosophers and on the study of philosophy itself. Indeed, long though this biography is, the author offers us no final reflections on Russell's position as a mathematician and philosopher.

It may well be that Russell learned more from continental European philosophy than he contributed to it, and that when we seek to establish the intellectual and cultural contours of this century his own thought will be related primarily to the intellectual and cultural history of this country and of the US. When he received the Nobel Prize it was for literature, and his own *History of Western Philosophy*, a best seller from the moment of its publication, must be judged in very different terms from *Principia Mathematica*.

Of his eminence—a Victorian concept—there was no doubt. Nor did he himself doubt it. Walking with a friend in Trinity, Cambridge, one day, he pointed to the marble busts on pedestals, waved his hand as he passed the last one, and said, "I shall be here one day." □

The Life of Bertrand Russell. By Ronald W. Clark. Pp. 766. (Jonathan Cape and Weidenfeld and Nicolson: London, 1975.) £6.95.

Controlled drug use

Concepts in Biochemical Pharmacology, Part 3. Edited by J. R. Gillette and J. R. Mitchell. (Handbook of Experimental Pharmacology, New Series, Vol. 28/3.) Pp. xxviii+480. (Springer: Berlin and New York, 1975.) DM 198; $81.40.

MANY powerful drugs are now available and if they are to be used to best effect we need knowledge not only of how they act on the body, but also of how the body deals with the drug; both aspects are equally important in deciding how we use the drug. Important questions are: how much do we give? When? How? And to whom? This book is concerned with answering those questions. Work in this important branch of pharmacology has greatly expanded in the past decade, and although several texts have been published none are quite as detailed, or complete, as the volume of which this book constitutes the final part. In contrast to the two earlier parts, which were concerned more with methodology and general principles, this deals in detail with practical applications. Throughout, the editors have sought contributions from experts who have generally succeeded in combining the basic concepts with considerations of their clinical implications.

The first two chapters deal rigorously but comprehensibly with the mathematics of drug disposition. The second in particular relates the various models which have been developed to what actually happens in the body, with a full discussion of the importance of regional blood flow and protein binding. Subsequent chapters analyse factors which lead to individual variation in drug disposition (age, disease and genetic factors) and to variation within an individual (the route of drug administration and the form in which the drug is given). A section on drug interactions starts with a clear discussion of possible mechanisms and includes two interesting chapters which cover areas where beneficial interactions may occur: cancer and antimicrobial chemotherapy. The disadvantages of using antibiotics in combination are also stressed. A final section deals with some aspects of drug toxicity, particularly the role of chemically reactive drug metabolites in causing tissue damage.

The book achieves the same high standards of both content and presentation as its companion parts and together they constitute a most valuable reference work. Its detailed and rigorous approach make it most suited to clinical pharmacologists and research workers, particularly those involved in drug development. At the same time, a lot of what it has to say could be directly translated into prescribing practice, and it is to be hoped that it will reach a much wider readership among the medical profession.

D. G. Haylett

Textbook of psychopharmacology

Psychopharmacology: a Biological approach. (Series in Experimental Psychology.) By Robert. A Levitt. Pp. x+502. (Hemisphere: Washington, June 1975. Distributed by Halsted Press.) £9.45.

THE world still needs honest jobbing textbooks, and it is within that genre that the present volume takes its place. In the introduction, Professor Levitt describes the origins of this text as being material he prepared for a graduate seminar: seven of his students became sufficiently enthused subsequently to help in the further development of certain chapters, and they now appear as authors or co-authors. The result of this admirably cooperative venture is a text which is business-like and straightforward, but at the same time has about it a special feeling of enthusiasm. One may suspect that in this particular set of seminars no-one fell asleep.

The reasonable demands of any textbook should be that the facts are right, that the essential matters under treatment are adequately covered, and that the presentation is lucid. This book largely meets these basic expectations. The additional problem which an undergraduate text on psychopharmacology has then particularly to meet is the decision as to where the subject shall be circumscribed: neurophysiology, neuroanatomy, learning theory, clinical psychiatry, endocrinology, are among the obvious neighbouring states. In these borderlands it is difficult to find the balance between offering too much or too little contextual explanation and discussion, and in this regard the present text is not altogether successful. It is in these border areas that the student may be lost or mislead. For instance, in respectable circles amphetamines are not these days prescribed for treatment of depression. Involutional depression is not a synonym for endogenous depression. More importantly, there is an invitation to sad unawareness in the declaration that "Our position is that mental (behavioural) disorders are the sole result of either learning or physiological dysfunction, or some combination of the two". Indeed, in one section a "simplistic overview" is boldly offered as a good thing.

This is in many ways an attractive book, but that lively seminar material has not been entirely successfully transmuted into a rounded textbook of psychopharmacology.

Griffith Edwards

Action of antitumour agents

Antineoplastic and Immunosuppressive Agents. Part 1 and 2. (Handbook of Experimental Pharmacology, New Series. Vol. 38/1 and 2.) Edited by Alan C. Sartorelli and David G. Johns. Vol. 1, Pp. xxiv+762; DM258; $105.30. Vol. 2, Pp. xxxii+1067; DM 278; $119.80. (Springer: Berlin and New York, 1975.)

THIS two-volume book comprises a series of reviews, 79 in all, which give as wide a coverage of the nature of antitumour agents and their action as has yet been seen in one work.

The general title might suggest that antineoplastic agents and immunosuppressive agents are different groups, whereas the latter are in practice drawn from the former. Only three chapters (Part I) are devoted specifically to immunosuppressive agents but the coverage is adequate; moreover, the immunological properties of agents are discussed in subsections in other chapters.

Part I contains the more general articles. It includes chapters on the rationale of drug design, the choice and use of agents, and drug resistance. The editors have also wisely included reviews on related topics: cell kinetics, cell growth regulation, radiation, immunotherapy, and pharmacology in the broadest sense (organism to molecule). Part II is devoted to a detailed treatment of various agents including hormones.

The team of well-known contributors assembled by the editors virtually ensured an authoritative compendium, packed with information, marked by a lack of uniformity in chapter organisation which should worry none but the perfectionist. Most of the contributions were probably written three or four years ago but a time lag is unavoidable in the preparation of such a work. New agents of real value do not emerge very often. It is therefore certain that those concerned with the fundamental aspects of drug action will find this handbook a valuable reference work for years to come. It is equally certain that the high price will almost totally exclude this meritorious work from private bookshelves.

J. A. Stock

[illegible] Upper Circular Road
[illegible]

Vector–parasite

Invertebrate Immunity: Mechanisms of Invertebrate Vector–Parasite Relations. Edited by Karl Maramorosch and Robert E. Schope. Pp. x+365. (Academic: New York and London, June 1975.) $16.50; £7.90.

THIS is a collection of 24 papers by 29 authors given at a 'workshop' held in Bethesda, Maryland, in April 1974. It does not provide a complete or a balanced account of the present state of invertebrate immunology, but some of the contributions are useful as quarries for information or because they attempt a judicious review of particular topics.

Seven papers are grouped under the heading "Invertebrate Gut as a Barrier to Invading Parasites", an excellent subject, worthy of fuller discussion than it gets. A preliminary account of the midgut epithelium loses its way among electron micrographs of cell junctions, but T C. Orihel shows how the peritrophic membrane of Diptera can limit infection by microfilariae and trypanosomes All the other papers are concerned with viruses, particularly with factors affecting virus infection of the gut wall of vectors, principally insects. There is valuable material here for the specialist, but some of it is difficult to extract from the language. A surprising gap is the absence of any account of the fate of ingested bacteria.

"Analysis of Invertebrate Immunity" is not a appropriate heading for the next seven papers, some of which contain much that need not have been said again. An admirable contribution is that of R. S. Anderson, who reviews biochemical and other characteristics of phagocytosis by invertebrate cells *in vitro* and compares them with vertebrate systems. A. R. Barr discusses evidence for the genetical control of invertebrate (mainly mosquito) immunity to microfilariae and plasmodia.

Part III, comprising five papers on components of the haemolymph, includes a useful review by M. R. Tripp on humoral factors in molluscan immunity, and another by G. R. Wyatt on some biochemical features of insect and arachnid blood. J. S. Chadwick gives a detailed account of changes in the haemolymph related to infection or to induced immunity.

Another odd heading, "Vector Destruction", groups the last five papers. They include a largely pictorial account of encapsulation and melanisation in insects by A. J. Nappi, and a discussion by L. M. Riddiford of some of the available evidence about hormones of insects in relation to their parasites.

Like most publications of its sort, this book is very uneven. Much that is important and useful is brought together in some of the contributions but the volume contains a lot of dead wood. Even specialists will have to use it circumspectly. The strong bias towards insects and molluscs as hosts and towards viruses, microfilariae and protozoa as parasites sorts ill with the claim that. "This book provides the first modern, integrated presentation of phenomena and mechanisms pertaining to immunity in invertebrate animals". All three adjectives can be challenged.

George Salt

Catalytic bodies

The Mathematical Theory of Diffusion and Reaction in Permeable Catalysts. By Rutherford Aris. Volume 1: *The Theory of the Steady state* Pp. xvi+444 £13 net. Volume 2: *Questions of Uniqueness, Stability and Transient Behaviour.* Pp xiv+217. (Clarendon: Oxford; Oxford University: London, 1975.) £8 net.

IN spite of the steady progress that is being made in improving the performance of catalysts, the problem of predicting the behaviour of a permeable catalyst, in which diffusion plays a role, remains an unpredictable and sometimes costly occupation. It is timely, therefore, that an excellent text on the mathematical theory, and its applications in chemical engineering and biology, should appear. Volume 1 deals comprehensively with the steady state behaviour of a catalytic body. Volume 2 establishes the links between uniqueness, stability and transient behaviour of reacting systems in which diffusion is involved.

Chapters 1 and 2 lay the physicochemical basis of the theory and derive the general equations of diffusion and reaction, assuming the catalytic body to be a homogeneous continuum. This assumption has been called into doubt recently and there is a need for further experimental work to test the mathematical models.

Chapter 3 considers the single reaction in an isothermal catalytic body. There are two particularly interesting sections concerning the development of approximate solutions based on singular perturbation analysis and variational methods. Chapter 4 extends the study to non-isothermal behaviour. After a brief survey of numerical methods, the main theme, which deals with the complex interactions of diffusion and reaction, unfolds beautifully and we are left quite breathless with the staggering prospect of possibly an infinite multiplicity of the steady state, although thankfully the conditions are rather extreme in practice. The concluding section on asymmetrical solutions is also potentially very important.

Chapter 5 is perhaps more low key but nevertheless focuses on the important area of multiple reactions. There is certainly much scope for ingenuity here in formulating non-uniform catalysts to improve yield. Chapter 6 deals with questions of existence and uniqueness of the steady state, followed by an outstanding discussion on stability in which important theoretical results are presented. The concluding chapter presents several fascinating aspects of the transient behaviour, such as deliberate periodic operation to improve selectivity, catalyst decay and so on.

The main objective of this book, which is to bring together many results on the theory of diffusion and reaction, is achieved with a style and grace which we have all come to recognise as pure Rutherford Aris. These two volumes should certainly be on the bookshelf of any aspiring chemical reaction engineer. His predictions may still be wayward but now at least he has the doubtful consolation of a hundred and one possible explanations.

D. L. Cresswell

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Structure–property relationships

Structure–Property Relations. By R. E. Newnham (Crystal Chemistry of Non-Metallic Materials, Vol. 2) Pp. x+234 (Springer: Berlin, Heidelberg and New York, 1975.) DM72; $31.00.

THIS book essays a formidable task in its purpose of relating material properties and structure, in particular in demonstrating the application of some concepts in crystal chemistry to technologically important materials. The first chapter briefly reviews the principles of symmetry and crystal physics and emphasises the importance of such considerations in determining whether a given property can be developed by a crystalline material with a particular regularity of atomic architecture. In the remaining six chapters properties falling within the general fields of electronic transport, thermal properties and ion transport, ferroelectrics and other ferroic materials, optical materials, magnetic materials and materials with useful mechanical properties are described, in each case an outline of the physical principles on which the property is founded is given together with illustrations of particular structural configurations which promote the mechanisms of electronic and atomic movements responsible for the property.

To sketch in even the essentials of such a broad canvas in so short a volume demands economy of presentation Newnham's text is compact, at times terse, so that it requires sustained concentration and careful study, even by a reader with some familiarity with the contents; this effort, however, often has its reward in an increased insight into a particular phenomenon. Inevitably, too, there must be occasions when the brevity demands simplistic statements which would be qualified in fuller and more rigorous accounts. These shortcomings are overcome by the references to other books and original papers which supplement each chapter and which must be accepted by a reader as an essential part of the presentation.

To assess the value of any book critically one should have a clear concept both of its objectives and the readership to whom it is directed, and it is on this latter aspect that doubts persist: the author's own preface provides no real clue, though perhaps there will be clarification when Volume 1 of the same series is published later This book cannot be regarded as any kind of course text, though lecturers may find some parts stimulating when preparing their own course presentation. To describe it as a review is probably misleading in that the limited coverage of any given topic requires that a practising scientist must go on to broader and deeper considerations. Its appeal is greatest if it is judged as some kind of hybrid between an advanced course text and a strict scientific review. In this light it could have a value on the library shelf for both inexperienced and experienced graduates, with sufficient background familiarity in materials studies, wishing to enter a new, unknown area of structure–property relations; but it must be emphasised most firmly that the character of presentation assumes a wide general knowledge of crystalline matter possessed by many materials scientists but certainly not by others, such as conventional engineers. Professor Newnham has made an honourable attempt to solve a difficult problem, but it may well be that a much more expanded treatment is essential; certainly it is a remedy that one is hesitant to recommend with the soaring costs of book production, already apparent in the price of this slim volume.

Peter Gay

Genetic combination

Genetic Structure and Function. By P. F. Smith-Keary. Pp. xvi+368. (Macmillan: London and Basingstoke, March 1975; distributed in the US and Canada by Halsted Press.) Boards, £7.95; paper, £3 95

Genetic Structure and Function represents an attempt to encompass within a single volume an account of genetics at the molecular level, extending from classical genetic analysis to molecular biology. As the author observes in his preface, there are many books of general genetics, but they are all orientated towards the Mendelian approach, and the books on molecular genetics in general tend to start from a more biochemical position. This book is intended "to achieve a better balance between the genetic and the biochemical evidence"

After a brief introductory chapter, the next three chapters deal with the nature of the genetic material as nucleic acid, the structures of bacterial and viral genomes, and eucaryotic chromosomes It is a straightforward account. the only deficiency of which is a failure to progress to the organisation of eucaryotic chromatin in terms of its components (such discussion is limited to a page).

The next group of chapters essentially concerns traditional genetic analysis in eucaryotes, fungi, and bacteria and bacteriophages. The first of those chapters develops the principles of genetic mapping, a concern which is taken up in detail for each system in the succeeding chapters. This discussion is competently written if somewhat unexciting in view of the subject matter.

The remaining half of the book is devoted to what might be described as molecular biology, with chapters on the nature of mutation, the relationship between genes and proteins, the genetic code, transcription and translation and their control; a chapter on recombination is the only part of this which relates directly to the earlier material. This again is a well written and clear account, although necessarily of some brevity.

The final two chapters cover a variety of topics: suppression of mutations in bacteria, position effects in maize and *Drosophila*, gene expression in differentiation. These are the weakest; and the last chapter, given the broad title "Genetic Organization and Function in Eucaryotes", provides so condensed an account of its subject that it is difficult to believe readers will gain much from it. Discussion of the components of eucaryotic chromosomes and of the processes of transcription are perfunctory to say the least.

In view of the author's stated aim to provide a more balanced account of molecular genetics, perhaps it is surprising that the book commences with molecular descriptions, and then passes through classical genetic analysis to deal with molecular biology; a more logical development might have been to progress from a starting point of Mendelian genetics. As an introductory account, however. this book has much to commend it: it provides a complete discussion of all topics pertinent to procaryotic molecular genetics, in spite of its broad scope it is accurate and rarely oversimplified, it is well written and easy to follow, and the bibliography is sensible.

Dr Smith-Kheary suggests that this book will be useful to second and third year undergraduates (in British terms): but one may feel that a more detailed account will usually be required by the third year Indeed, given that molecular genetics is a subject of some sophistication, usually approached only after some knowledge of classical genetics has been attained, it is not surprising that separate books are usually written on classical and molecular genetics The weaknesses of this book clearly are imposed by the attempt to provide a complete account covering such a breadth of material; one wonders if students would not in fact benefit from the more extended discussion possible when more than one book is used

Benjamin Lewin

obituary

Vladimir Nikonovich Starovskii, a leading Soviet statistician and until recently Head of the Central Statistical Board of the Council of Ministers of the USSR, died on October 20 at the age of 70.

Starovskii, was born at Pomozdino, in what is now the Komi ASSR. At the age of 18, he enrolled at the University of Moscow, and, while still a student, began working for the Central Statistical Board After graduting in 1926, and taking a postgraduate course at the Institute of Economics, he combined his duties at the Central Statistical Board with an academic career, holding a number of teaching and professorial posts, and publishing several major works on statistics, including *Parameters Characterising Correlation Connections, The Sampling Method, Theory of Methematical Statistics,* and *Basic Problems of Population Statistics*. In 1940 he received the degree of Doctor of Economic Sciences, and in 1957 he was elected a Corresponding Member of the Academy of Sciences of the USSR During his long career, Starovskii became known as one of the main organisers of governmental statistics in the Soviet Union For his services in this field, he received numerous medals and prizes, including the Order of Lenin (three times), the Order of the Red Banner of Labour (twice) and the title of Hero of Socialist Labour

Aleksandr Pavlovich Vinogradov, a vice-president of the Soviet Academy of Sciences, died on November 16, 1975.

Vinogradov was born on August 9 (Old Style), 1895 in St Petersburg In 1920, he enrolled in the Military Medical Academy, and the Faculty of Chemistry of Petrograd University, graduating in 1925, to become an Instructor at the Military Medical Academy, and, simultaneously, an assistant at the Biogeochemical Laboratory of the Soviet Academy of Sciences. From this time on, his career was closely associated with the Academy, he became a Corresponding Member in 1943, and a full member in 1963. From its foundation, in 1947, he was director of the "V. I. Vernadskii" Institute of Geochemistry and Analytical Chemistry of the Academy, and in 1967, he became one of the Academy's vice-presidents.

Vinogradov's numerous published works range from biology to geochemistry. He is particularly noted for his work on the distribution of elements in soils and bedrock, on the origin of ores, and for his hypothesis on the formation and evolution of the terrestrial crust and oceans. He also did important work on the geochemistry of the Moon and planets, and showed considerable interest in applications of atomic energy (in 1957 he read two papers to the Geneva Conference on the Peaceful Uses of Atomic Energy).

For twenty years, Vinogradov was head of the department of Geochemistry at Moscow State University, and is considered to be the founder of Soviet geochemistry. He was a winner of the Lenin and Stalin (now State) Prizes, and was awarded a number of high Soviet honours, including the Order of Lenin (three times), the Order of the Red Banner of Labour (twice) and various medals. **Vera Rich**

Torkel Weis-Fogh, Professor of Zoology at Cambridge, died on November 13 at the age of 53. He was a citizen of Denmark and a Fellow of the Royal Danish Academy, and before his election to the Cambridge chair he had been Professor of Zoophysiology at Copenhagen.

His first academic appointment was as assistant in research to August Krogh who had begun, after his retirement, a study of the metabolism of the desert locust Weis-Fogh took up the flight mechanism and with the collaboration of Martin Jensen, an engineer, he published several papers on the aerodynamics of locust flight. From aerodynamics he turned to the physiology of the locust flight muscle, and out of this interest in contractile systems was later to come the work, still in progress, on the vorticellid spasmoneme. Following up a difference between the apparent and the expected efficiencies of locust flight muscle he was led to a small pad of protein in the wing articulation, which he showed to have the properties of an almost perfect rubber After coming to Cambridge in 1966 he went back to aerodynamics to investigate the flight of very small insects, whose ability to fly at all was unexplained, and out of this came the discovery of a hitherto unknown means of generating lift. These last two examples admirably illustrate his characteristic approach to a problem—analyse it and quantify it on the basis of current theory and then seek out the reasons for any anomalies.

Arising from his discovery of resilin, he became interested in the fine structure of the insect cuticle and the manner in which it is laid down, and also in the basis of the long-range elasticity of the vertebrate protein elastin. Yet he found time to spare from his own research to initiate important new inter-disciplinary enterprises. One of these was the use of electron microprobe X-ray spectrophotometry for the analysis of micron-sized areas in deep-frozen hydrated sections, a technique which will undoubtedly advance the work of many biological departments in Cambridge. And at the time of his death he was preparing, in collaboration with Sir James Lighthill, to launch a major project in biological fluid dynamics.

Weis-Fogh was held in the highest regard by zoologists and physiologists the world over, but nowhere more than in his own department where regard was compounded of affection as well as admiration. **J. A. Ramsay**

International meeting

January 8–9, **Food supplies: outlook for Britain,** UMIST Manchester (Royal Society of Health, Conference Department, 13 Grosvenor Place, London SW1X 7EN, UK).

Reports and Publications

Other countries

United States Department of the Interior· Geological Survey Bulletin 1309 The Geologic Story of the Isle Royale National Park. By N King Huber Pp v + 66. Water Supply Paper 2032 Ground Water in the Corvallis-Albany Area, Central Willamette Valley, Oregon. By F J. Frank Pp ix + 48. (Washington, DC Government Printing Office, 1974 and 1975.) [1310

World Health Organization, Technical Report Series No 573· The Veterinary Contribution to Public Health Practice Report of a Joint FAO/WHO Expert Committee on Veterinary Public Health. Pp. 79. Sw. fr. 8, No 574. Pesticide Residues in Food. Report of the 1974 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues Pp 37 Sw. fr. 6. (WHO Geneva 175) [1310

United States Department of the Interior Geological Survey Bulletin 1387 Checklist of North American Late Paleozoic Coral Species (Coelenterata, Anthozoa) By William J Sando, Pp iii + 36 70 cents Water-

Supply Paper 2035: Geohydrologic Reconnaissance of the Upper Potomac River Basin. By Frank W. Trainer and Frank A. Watkins, Jr. Pp. v + 68. Water-Supply Paper 2093: Quality of Surface Waters of the United States, 1968. Part 3 Ohio River Basin. Pp. x + 309 $1.75. Water-Supply Paper 2121. Surface Water Supply of the United States, 1966–70. Part 7: Lower Mississippi River Basin Vol. 2: Arkansas River Bason. Pp ix + 931. $6.15. Water-Supply Paper 2140. Ground-Water Levels in the United States, 1968–72. Northeastern States. Pp. v + 207. $1.75. Water-Supply Paper 2147 Quality of Surface Waters of the United States, 1969. Part 8: Western Gulf of Mexico Basin. Pp. xi + 567. $4. Water-Supply Paper 2156: Quality of Surface Waters of the United States, 1970. Part 7: Lower Mississippi River Basin. Pp. xii + 636. Professional Paper 858–B: Carboniferous Foraminifera and Algae of the Pennsylvanian of Wyoming. By Bernard L. Mamet. Pp. iii + 18 + 2 plates Professional Paper 861. *Chesapecten*, a New Genus of Pectinidae (Mollusca. Bivalvia) from the Miocene and Pliocene of Eastern North America. By Lauck W. Ward and Blake W. Blackwelder. Pp. iii + 24 + plates 1–7. $1.45. Professional Paper 868 Post-Paleocene Tertiary Rocks and Quaternary Volcanic Ash of the Wet Mountain Valley, Colorado. By Glenn R. Scott and Richard B. Taylor. Pp. iii + 15 + plate 1. $1.55. Professional Paper 883 Discrimination of Rock Types and Altered Areas in Nevada by the Use of Erts Images. By Lawrence C. Rowan, Pamela H. Wetlaufer, Alexander F H. Goetz, Fred C. Billingsley and John H. Stewart. Pp. iv + 35. $1.55. Professional Paper 900 Geological Survey Research, 1974. Pp. x + 349 Professional Paper 912: Petroleum Geology of Naval Petroleum Reserve No 1, Elk Hills, Kern County, California. By J C. Maher, R. D Carter, and R. J Lantz. Pp viii + 109 + 25 plates. (Washington, DC. Government Printing Office, 1974 and 1975.) [1410

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nature

December 25, 1975

New era of firmer control in French science

SUPPORT for science marks time. Universities live on budgets which in some countries, the UK included, have recently shrunk in real terms. Government interest in science is, at best, lukewarm. Students decide in large numbers that a career in science is not for them. Everywhere the scientist finds himself less loved than he used to be. Almost everywhere, that is; some interesting things are happening in France.

During the Gaullist period, French science grew at a healthy rate—in 1967, for example, research and development accounted for about 2.1% of the GNP. In subsequent years this figure declined; by 1974 it was down to 1.7%. Now President Giscard d'Estaing seems to have made a positive decision, after an eighteen-month policy review, to go for growth again. It is likely that, having seen that some other comparable industrial countries are spending rather more than 2% of their GNP on research and development, he will attempt to bring France up to that level again. A recent council at the Elysée Palace—the second in a year—wrestled with the problem of how to bring about the growth.

The President is not ordering this expansion for the sake of increasing prestige. He sees that by the year 2000 France's manpower resources will not have risen substantially, and so it will be increasingly relying on the export of technological expertise to keep its place among the rich nations. It is largely for the maintenance of France's intellectual resources to do just this that Giscard d'Estaing wishes to increase his support for science. The new policy can be seen to have two distinct aspects: reorganisation of research priorities, and the creation of more employment opportunities.

In the past, various organisations have sprung up to carry out specific tasks; they have included IRIA (for Information Processing and Automation), CNES (for space research), CNEXO (for ocean exploration) and ORSTOM (for overseas research). As time progressed the missions of these bodies changed somewhat in response to both internal and external pressures. But they had no monopoly on research in their own field, and friction developed as questions of 'who does what' arose. Now it is proposed that consultative committees be established to act as buffers between the individual organisations and the Minister for Industry and Research, M d'Ornano. These committees will, it is expected, make it more difficult for scientists to act both as appellant and judge in deciding what projects should be undertaken. M d'Ornano has made no secret of his wish to have firmer control over the work done in the laboratories, and this new mechanism will undoubtedly help him, although it may not be well received by the research workers' unions, who have come to expect increased participation of workers in decision-making on matters of policy.

On the other hand, the unions will probably be fairly satisfied by improvements being made in terms of employment. In recent years a growing number of French scientists have found themselves having to work on short-term contracts, as tenured posts have dried up. This problem is now to be tackled; many thousand posts for researchers and technicians which at present carry little security are to be converted, in the next five years, into more stable positions. And, as an incentive to young students to work in fields which the government judges to be of priority, up to 1,500 studentships will be created, paying 2,000 francs per month for the second and third years of postgraduate thesis work.

There are risks in this, of course. Fundamental research could well suffer if the mood that research should satisfy national goals becomes too strong. It would be difficult to demonstrate that the UK had, in the post-Rothschild period, lost out on fundamental research while attempting to stimulate more applied research. But the universities of France do not have as strong a tradition as do the British ones of acting as the guardians of fundamental research.

A justifiable case for support?

The passing of the old year next week will be celebrated with no little fervour by the UK computer industry. The economic recession triggered by the oil crisis really hit hard during 1975, so much so that survival rather than growth became the priority, and the short-term objectives of maintaining profitability and, even more important, good cash-flow, tended to override all other considerations. Nicholas Enticknap reviews the state of the industry.

MOST British computer companies can look back on the year with satisfaction. The most important of them, International Computers Ltd (ICL), has just reported its trading figures for the financial year ending September 30, and these show a 20% increase in turnover, less than half of which is accounted for by price rises, and a 36% increase in profit after tax. Another leading company, Data Recording Instrument Co., continued to increase its exports and was able to set up a new subsidiary in France. Two of Britain's largest computer service companies, CMG Computer Management Group and Logica, both increased their turnover by more than 50% though pre-tax profits were less than hoped for.

On the debit side, Core-Computer Related Equipment, a distributor of imported computer equipment, went into liquidation last month. And a little earlier, Process Peripherals, a company which specialises in specially strengthened data storage devices, ran out of money, in spite of a large and healthy order book. The company's assets have, however, been bought by a British electronic company, Gresham-Lion, and this has enabled Process Peripherals to carry on.

Looking at the UK industry in a broader context, though, it is still nowhere near to supplying the needs of the country's users, far less becoming a net exporter. The most recent year's figures supplied by the Department of Industry are for 1974; they show exports of £210 million and imports of £346 million, a net deficit of £136 million Total sales of the industry were £517 million The figures for the first six months of 1975 are similarly depressing.

But these figures, bad as they are, actually flatter British industry, for they include sales of equipment manufactured in Britain by foreign manufacturers. Four of the top five American computer companies, IBM, Honeywell, Burroughs, and NCR, have factories in the UK, most of them located in Scotland These factories do, of course, contribute to the balance of payments and provide employment, but the profits end up in American rather than British pockets.

Market shares provide another measure of the success of British industry, though admittedly a controversial one as the arguments over the definition of the market and the best method of valuation are endless. According to ICL executives, who can reasonably be expected to be as favourable to ICL as anybody, their company is maintaining a steady 38% share of the total value of all computers installed in the UK. No other British company has a significant share, so well over half the computer equipment in use in Britain was made by foreign companies.

This again, unsatisfactory though it is, is flattering rather than the reverse, because, for example, many of ICL's computers use disk drives designed and manufactured in America, and these can account for a substantial proportion of a computer system's total value. Nonetheless ICL, which was formed by merger of several different computer companies in 1968, has done well; it has a larger share of its home market than any major computer company, other than IBM, albeit in somewhat artificial market conditions. IBM is the giant of the industry, and has sold between 60 and 70% of the total value of all computers installed worldwide—only in Britain and Japan of the industrialised countries does it have less than 50%.

ICL sells 'mainframe' computers, which are general purpose systems ranging in value from around £30,000 to several millions. Another class of computer, the minicomputer, is normally used for one or two specific tasks such as control of an industrial process, but is increasingly being adopted for commercial use. Prices range from around £1,000 up to around £100,000, but nonetheless, minicomputer business is big business—the leading manufacturer, Digital Equipment Corp., has a turnover of $500 million.

Britain's record in this area is little short of appalling. There has been no attempt to merge the number of small companies competing with each other into one cohesive unit, with the result that none of them has become a major force, and most of them have suffered financial disaster. Ferranti, whose troubles are well known, is the biggest producer, with a manufacturing volume of around 30 systems a month. This compares with over 100 produced by the Swedish company Datasaab, and volumes ranging from 350 to 500 for the leading American manufacturers.

This record is not due to poor design, for British minicomputers have generally received high marks for technological expertise and innovation in independent surveys. It is primarily due to the limited size of the home market, which simply cannot support a large number of companies. This means that development costs are spread over a smaller number of units, so prices are higher and contracts are lost. Furthermore, there is no preferential purchasing policy by government departments to compare with that enjoyed by ICL.

The question of government involvement in the computer industry is a perennial source of concern. There can be no doubt that the government should formulate a policy. The net deficit in the balance of payments should in itself be a matter of national concern, the more so as sales of computer equipment are projected to grow by 15% a year in the UK into the indefinite future. The types of computer equipment that have the biggest growth potential—minicomputers, terminals and microcomputers—are all dominated by American companies to an even greater extent than the mainframe business, so the trading imbalance is likely to get much worse unless corrective action is taken.

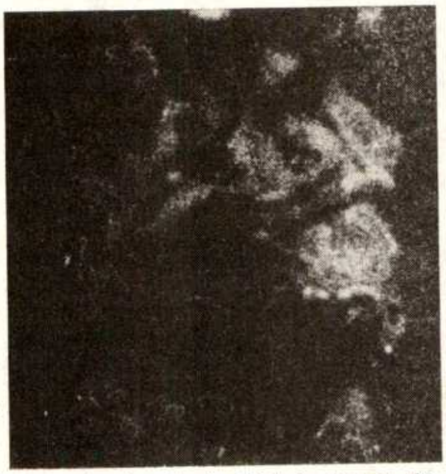
In spite of this, the government has never had a planned policy for the computer industry, and has merely responded to individual problems when sufficient pressure has been exerted. This is extraordinary considering the enormous sums of money spent on the motor industry this year in the face of advice from government bodies that there is little prospect of a return on this investment. The bills for Concorde have come to around £1,500 million, spent in the cause of developing new technology Yet the computer industry both embodies new technology and offers hope for a return on investment, but has only received government funds to the tune of around £50 million since the formation of ICL.

This £50 million is largely accounted for by an investment-free loan of £40 million to ICL for development of its latest range of computers, the 2900 series. Other smaller sums have been made available to a number of companies through the National Research Development Corporation and the National Computing Centre, but these have all funded a maximum of half the total cost of each development project. The government does help in one other way, by requiring its own departments to buy British (which means ICL) when purchasing any computer with more than a specified amount of computer power.

One result of this lack of support is to induce British companies to adopt an "if you can't beat them, join them" policy. ICL has joined in a three-sided agreement with two American companies, NCR and Control Data Corporation, covering the development and manufacturer of peripherals. Data Recording Instrument Company has similarly made agreements with various North American companies concerning the supply and manufacture of disk drives.

It is depressing to move into 1976 without any sign of a change of attitude from the British government. Certainly none could be detected in the speech made by Eric Varley, Secretary of State for Industry, at the opening this month of the National Computing Centre's new headquarters. Still, it could be worse. One of the saddest events of 1975 was the collapse of Unidata, a pan-European computer manufacturer formed from the French government company Compagnie Internationale pour l'Informatique, and from the computer subsidiaries of Philips of Holland and Siemens of Germany. ICL was at one stage under considerable pressure to join this triumvirate, but resisted, and consequently remains alive and well—and profitable. □

Nessiteras skeptyx



WHEN the Loch Ness Monster ostensibly raised its ugly head (see picture) with the support of Sir Peter Scott, widespread public interest was inevitable. What was far less certain was the reaction of the scientific community. As it turns out, Scott and his colleague Robert Rines must be reasonably content.

Their original plan was to present at a two-day scientific symposium the full evidence which they felt supported the existence of large animals in Loch Ness. The outcome, they hoped, would at least allow the existence of the animal to be given the benefit of the doubt. They also wanted it officially named in order to have it legally protected as an endangered species. An attack of cold feet gripped the organisers of the symposium, however, and they withdrew their support.

The only forum left for the presentation of evidence was a meeting in the Grand Committee room at the House of Commons—a room, some would say, that is steeped in the history of monstrous debate. It was held on the evening of December 11 before representatives of the public, the press, and the scientific community, along with Members of Parliament understandably seeking relief from the second reading of the Armed Forces Bill, which was making Mr Wellbeloved less endearing then Nessie.

The bulk of the meeting was devoted to presentations by members and associates of the Academy of Applied Science—a 'non-profit, scientific and educational corporation' lodged at the Boston home of its president, Robert Rines. The academy has been investigating Loch Ness every summer for the past five years.

The optical and sonar systems used and the site chosen for the attempt to obtain underwater photographs of the monster were both presented in dazzling detail. Much was then made of the 1972 photographs which, after computer enhancement, showed 'flipper-like objects'. But when it came to the expected climax of the 1975 photographs, Rines' presentation was so modest that he nearly blushed. No attempt was made to identify the objects shown. That, said Dr Rines, was for qualified biologists to do—not himself.

Three biologists handily placed on the platform then stated their beliefs that the photographs contained various images suggestive of flippers, a body and a head, all of which indicated the presence of large animals in Loch Ness. In that they were further supported by a statement from Professor A. W. Crompton of Harvard. None of the biologists, however, was prepared to try to identify the animal.

The discussion period that closed the meeting provided few fireworks. Most of the questions were on a scientist-to-scientist basis; they ranged from the problem of whether the animal would be air-breathing to queries about size controls for the objects photographed. Critical comments attained various levels. Hugh Fraser MP said that judging by his disappointing fishing experience in Loch Ness, the salmon population of 13 million claimed earlier in the evening was a gross exaggeration. He did not profer an alternative explanation of his performance, relating to the likely appetites of any animals in the Loch. David Attenborough suggested that the 1972 flipper-like objects might actually be the fin of a fish. And that, it seemed, was the cue for the heavy artillery from the British Museum to open fire.

Dr Gordon Sheals supported the view that the flippers were fish fins, and that their estimated size was "wildly" miscalculated. When that was disputed and further claims were made for the presence of a large animal, he decided it was time to broaden the attack. There was, he said, no evidence to support the view that the same object was shown in each photograph, nor to suggest that any of them showed a living object. Furthermore—and this was the body blow—it was most regrettable that *Nature* had published the article by Scott and Rines.

Another sceptic from the British Museum, Dr Humphrey Greenwood, conceded what several more sympathetic voices had already suggested—that there was a sufficient case for the evidence now to be published in full detail so that it could be properly scrutinised. The delighted Dr Rines clearly felt the battle was half won. He was at least being taken seriously.

Less serious was the response of the mass media. By and large the photographs were presented with a healthy dose of scepticism to accompany them and some astonishing artist's impressions of what they showed. The more enterprising journalists managed to winkle out people with something they thought approached a knowledgeable view. Unsurprisingly, the claims were as diverse as they were knowledgeable. One view was that the photographs actually showed parts of a Viking ship. Another, on television, was that the "head" was of a scuba diver wearing his breathing apparatus back to front. The grateful monster-following public also learned that the British Bacon Curers' Federation was to organise a hunt for Nessie by hot air balloon.

If the eventual truth of the monster-hunters' claims is still breathlessly awaited, at least they, though perhaps not their prey, have finally come in from the cold. That may be the epitaph on this episode. In the next one, the natural history of Loch Ness may even become a respectable line of study.

international news

Exit the Dragon?

by Roger Woodham

THE slight chance that the Dragon international high temperature reactor might be saved at the eleventh hour by the EEC Council of Ministers slipped away last week when the matter failed to come up for discussion at all. The decision taken the previous week in Brussels therefore stands, and the Dragon will be killed off—though not immediately dismembered—on March 31.

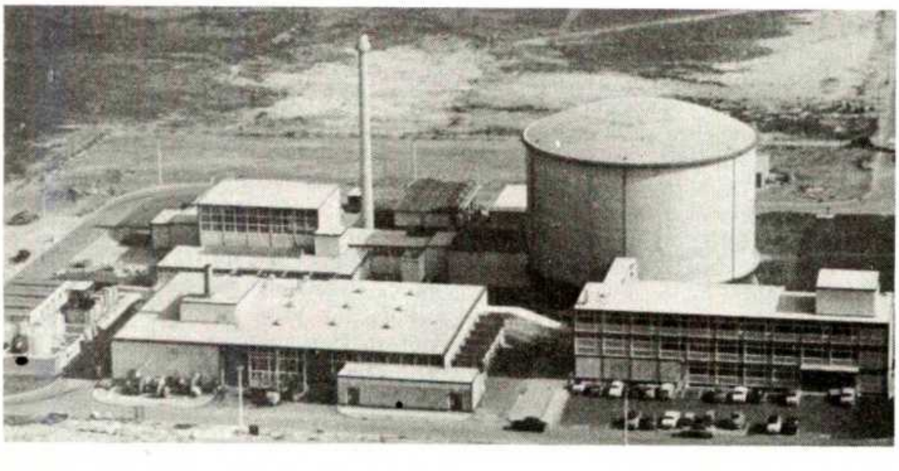
This brings to an end a three-month period during which optimism and pessimism over the future of Dragon fluctuated weekly, with the US Energy Research and Development Administration (ERDA) expressing interest in the project without putting any money on the table—or rather on the telex. Any UK agreement to continue the project eventually came to depend on some form of financial involvement by ERDA, and when this failed to materialise, the EEC, representing several of the participants in the project and therefore having the whip hand, decided to finish the project off.

The only real concession achieved in the past few weeks was a stay of execution of three months, from December to March. Fortunately the majority of the 300 or so people who work on Dragon are seconded from the UK Atomic Energy Authority (UKAEA) or its equivalent in the other member countries, or from industry, so they will simply return to their own organisations.

What happens between now and March 31? Certainly nobody is going to start taking the reactor to pieces—the UKAEA doesn't really know how to dismantle a reactor safely yet—and it will simply be mothballed. Late last week the European Parliament in Strasbourg criticised the decision and called for the reactor to be saved, and there is always the chance, of course, that ERDA, which by all accounts has been showing more definite interest even during the past week or so, may come up with a concrete proposal. But the possibilities all seem rather remote now.

Nothing definite emerged from a debate in the British House of Commons last week either. Replying to critical questioning, Alexander Eadie, Under-Secretary for Coal at the Department of Energy, said that the government was conscious of its special place as host, and did not want the project ended if other participants wanted it tot continue. But in that case, why not leave more time for alternative plans to be worked out properly?

It looks as if the Dragon on Winfrith Heath will stand as a monument to 16 years of work which eventually got out of step with the UK's nuclear aspirations. ☐



The Dragon reactor: now a monument?

University money plea

IN the politest way possible, the Committee of Vice-Chancellors and Principals of UK universities has asked Mr Fred Mulley, the Secretary of State for Education and Science, to put university finances back on to a firmer footing.

The recently issued statement "Universities in a period of economic crisis" asks the government to do three things:

- provide a planning perspective and a 'real terms funding commitment' extending beyond a year;
- assure universities that beyond the short-term crisis they will be able to resume development programmes with support "at a level in keeping with their national and international standing and commensurate with the burdens they have shouldered", and
- give a commitment that long-term planning and financing arrangements will be restored.

The committee reminds Mr Mulley that universities have been working on reduced resources in real terms for the last eighteen months and yet even so managed to increase student numbers by 7,000 in 1974 and 10,000 in 1975. The number of full-time students in British universities is now 263,000. More growth is forecast in the near future as the population of 18-year-olds has started to increase again and there is a "welcome surge" of applications from women.

Grants to universities are channelled through the University Grants Committee, and academic salaries comprise a little over 50% of university expenditure. Increases in salaries are guaranteed by the government, but the other half of recurrent expenditure (salaries of other staff, maintenance, supplies and so on) is compensated against inflation in rather a strange way. A costs index is used and the government may choose to make appropriate compensation in arrears.

In the calendar-year 1973 costs rose 12%, and the government chose not to supplement at all for the academic year 1974–75. Meanwhile cost inflation was running at 29% in 1974. As a result of deputations, the government later supplemented to the tune of £19 million for the year 1974–75 but, universities claim, this represented only a third of the sum necessary to maintain the grant in real terms.

In response to this universities had to make their own economies, by freezing staff posts (500 are now frozen), by letting non-academic staff go, and by saving of libraries, fuel, maintenance and so on. In terms of physical facilities, the universities now believe they have done all they can reasonably hope to, and in terms of staff cuts, they are now up to a student:staff ratio of 10:1. "Lasting damage would shortly ensue", they claim. ☐

Cottrell call for coal

A FORMER chief scientific adviser to the British Cabinet has involved himself in the continuing debate on the UK's energy prospects by publicly criticising the government for failing to appreciate the central importance of Britain's coal resources. In a letter to a leading newspaper last week Sir Alan Cottrell expressed concern that the country will face a serious energy deficiency when North Sea oil reserves are largely depleted in around 15 years time.

He called for an increased programme of government research into the extraction and efficient ultilisation of coal so that Britain can fill the post-1990 energy gap, arguing that the country cannot hope to meet its future energy requirements with nuclear power alone. It is estimated that by the year 2000, when nuclear output will only have doubled, Britain will face an energy gap equivalent to 20 times its present installed nuclear capacity.

Sir Alan says the present level of capital investment is insufficient to finance nuclear development on the necessary scale, and the recent cutback in the nuclear industry has resulted in a decline in the available constructional capacity. He adds that there is also a lack of public support for an expanding nuclear programme, and that a problem of uranium fuel shortage will arise.

He sees a fuller exploitation of British coal reserves as unavoidable, and argues that economic means of extracting coal from difficult deposits, almost certainly by remotely operated machines, must be developed; and that techniques for producing high-grade, low sulphur fuel oil and gas from coal must be advanced. Research into both of those problems is already well under way but Sir Alan says the programme will have to be stepped up if Britain is to come within range of its target in the time available.

The letter nevertheless endorses the recent enjoinder from the UK Royal Commission on Environmental Pollution that the government press ahead with the construction of a demonstration fast breeder reactor. And EEC policy regarding fusion reactors comes in for a timely sideswipe: according to Sir Alan, fusion reactors will not be ready for many years yet on the "prsently dilatory time scale". ☐

REGIONAL economic communities brought into being for the mutual benefit of their members will always experience difficulty deciding on the location of cooperative projects. The EEC is no exception, and last week provided a nice example of the problems involved.

The project was the Community's ambitious thermonuclear fusion programme for 1976–80, and the issue was the location of its acknowledged centrepiece, the large experimental rig called the Joint European Torus (JET). The result, at a Council of Ministers meeting in Brussels devoted to collaboration in scientific research, was that Italy blocked the programme—and the reason was that it wanted the project sited at the Nine's Joint Research Centre at Ispra in Northern Italy.

The European Commission, whose plans for this fourth thermonuclear fusion programme involve an expenditure over the four years of some £250 million, will not make a formal recommendation on the location until the new year, but has indicated a preference for Ispra.

Germany and France have had reservations, however, while Britain has pressed for the project to be sited at the Culham Laboratory of the UK Atomic Energy Authority. Unlike the other eight countries, Italy was not prepared to endorse the expenditure without a commitment to a location.

The Italian veto—for that is what it amounts to—marks a setback to a project which offers the possibility in the long term of an independent source of energy within the Community. Thermonuclear fusion is arguably a key to Community energy supplies beyond the end of the century, and marks one branch of research in which Europe is abreast of both the USA and the USSR. Herr Guido Brunner, the Brussels Commissioner responsible for scientific research, describing the delay as a "great error", warned that the Community's strong position in fusion research was in danger of being lost without an early go-ahead on the programme.

EEC diary

- Further signs of movement towards a European energy policy surfaced ahead of last week's Paris meeting between oil producers and consumers, for which the EEC finally acquired a mandate after the procedural difficulties that dogged the Rome summit meeting.

Apart from agreeing that average community oil consumption in the next two years should be a little below the 1973 level, a meeting of the Nine's foreign ministers received a progress report from the Commission on a directive under which member countries are obliged to keep an emergency oil reserve equivalent to 90 days' supply.

The requirement was fulfilled in June this year—when stocks were sufficient for 95 days' consumption. Although down from 101 days in January, the main problem was that the level was only for the Community as a whole. Some countries—West Germany, Italy, Holland, Belgium and Ireland—had failed to introduce the necessary legal measures to give effect to the directive, which came into force at the beginning of the year.

The Commission now intends to take legal action against these countries, although it admitted there were difficulties in stock building because of the large amounts of capital it immobilised and the balance of payments burden it imposed on the countries concerned.

- In the UK a recently published House of Lords Select Committee report criticises a Commission resolution calling for the standardisation of nuclear safety regulations within the EEC. The report, which comes only months after the committee strongly criticised Commission proposals for a Community energy policy, also attacks accompanying proposals that approval procedures for nuclear plant should be centralised in Brussels and that a body should be established to coordinate discussion of nuclear safety.

If the proposals are adopted, existing safety standards could suffer, the report says. The committee's chairman, Lord Lauderdale, said this was because the Brussels administration, with no real experts of its own, would preoccupy experts already working on safety problems.

The report says that to standardise safety proposals is impractical because "different types of reactor are in use, and design is developing". Standardisation will be "social rather than strictly commercial", and centralisation of approval procedures is seen as dangerous because "the ability to make direct representations to accessible elected representatives at a public enquiry is a valuable safety valve which would be lost". The establishment of a body to act as a catalyst for the international discussion of safety measures is according to the report, unnecessary in view of the plethora of organisations already in existence.

LEGISLATION to re-establish a science policy office in the White House, which has long been a goal of the scientific leadership in the United States, has encountered an unexpected roadblock in the Senate. Having swept through the House of Representatives a few weeks ago with scarcely a murmur of dissent, the bill is now stalled in three separate Senate committees chiefly because of growing disagreement about whether or not Congress should specify in detail the office's functions and areas of responsibility.

The essence of the matter is that Senator Edward M. Kennedy, who is the chairman of one of the subcommittees dealing with the bill, believes that the science policy office should play a strong role in military matters as well as domestic science policy affairs, and he wants to write strict provisions into the bill to ensure that is the case. The House-passed version, in contrast, would leave the President virtually sole responsibility for deciding how the office should function and what its areas of responsibility should be. Unless its role is spelled out in some detail, Kennedy is concerned that the office would be an ineffectual junior partner in the White House structure.

Kennedy's views are said to be shared by Senator John Tunney, who chairs one of the other subcommittees concerned with the legislation and, to a lesser extent, by Senator Frank Moss who chairs the third committee. That is a pretty solid front, and since the matter is very low in the consciousness of most other legislators, there will be little pressure for them to change their minds at least from within Congress.

The White House, however, is opposed to the notion that Congress should write a detailed prescription for an office which will be functioning at the President's elbow, and it strongly favours the House-passed version of the bill. The House members concerned with the matter are also of the opinion that the President should have considerable latitude in deciding how the White House staff should function, and have indicated that they are prepared to stick by their bill. They have argued, for example, that the influence of the science policy office will depend in large measure on whether or not it can function effectively in relation to other White House units and it should therefore be left to establish its own working relationships.

Although it is possible that the senators could compromise, and hammer out a measure acceptable to the White House and the House of Representatives, the prospects at present are uncertain. In any case, the start-up date for the office has receded considerably, and it now seems likely that it will be in place just as the Presidential election is in full swing. With President Ford's chances of re-election uncertain, it would be difficult to recruit good staff to the office, and nothing dramatic could be expected to emerge from it at least until the election is over.

Washington seen

by Colin Norman

● Another contentious issue is about to emerge in the bitter nuclear power struggle in the United States. On January 19, the Nuclear Regulatory Commission (NRC) will send Congress a report on the feasibility of grouping together nuclear power stations, and perhaps other nuclear facilities, on a few sites instead of scattering them around the country. According to a draft of the report, written by the NRC staff, such nuclear energy centres are deemed both economically advantageous and practical.

The matter is likely to be controversial, however, because such centres would raise some severe environmental problems, although they could help to mitigate some other concerns about nuclear power generation.

The study, which was ordered by Congress when it wrote the bill establishing the Nuclear Regulatory Commission, concludes that nuclear centres, with up to 20 power stations each, could cut the costs of building power plants by about 15%. Since individual plants are expected soon to cost about $1,000 million, such savings would be substantial.

But the environmental problems associated with nuclear energy centres would also be substantial. A 20-unit site, for example, would encompass about 40 square miles, compete for scarce water supplies with other users, such as cities and agriculture, and dispense so much waste heat into the environment that local weather patterns could be affected.

One frequently claimed advantage for grouping power stations, fuel fabrication and reprocessing plants together on the same site is that, when the nuclear industry begins using plutonium as a reactor fuel, having various facilities side-by-side will greatly reduce the risks that atomic bomb ingredients could be stolen in transit. But the study pours some cold water on that idea, suggesting instead that conventional safeguard procedures will be sufficiently effective to take care of the problem.

That particular statement is interesting in light of the fact that NRC has delayed making a decision on whether or not the nuclear industry should be allowed to recycle plutonium as a reactor fuel, precisely because it is not yet convinced that safeguard measures are adequate.

Be that as it may, the study is likely to generate a good deal of debate within Congress this year. It will also rekindle debate at the state level, since several state governments are considering adopting legislation to group power plants together.

● Spurred on by a highly critical report of the Food and Drug Administration's regulation of a ubiquitous food additive, Red Number 2, Senator Gaylord Nelson has proposed legislation which could force several food colourings off the market because of doubts about their safety.

Nelson, a liberal Senator from Wisconsin, who has long been a thorn in FDA's flesh, wants to repeal a provision of the food and drug laws which at present allows scores of food additives to be marketed while FDA collects data on their safety. The provision, which was written into the law in 1960, essentially allowed FDA 30 months to collect test data on additives that were then on the market, and determine whether or not they should be declared safe or banned. Fifteen years later, FDA has still not made up its mind about 90 colouring additives which were being marketed in 1960.

Among those additives is Red Number 2, which in 1973 was consumed at the staggering level of 1.1 million pounds in the United States. For years, there have been conflicting reports of health hazards associated with Red Number 2, including carcinogenicity, teratogenicity, and gonadal atrophy, and a few weeks ago, the Congressional General Accounting Office issued a sharply worded criticism of FDA's delay in deciding whether or not the additive is safe while children consume it in copious quantities in soda pop and candies. Nelson's bill would force FDA to make up its mind about those additives.

news and views

Antarctic jigsaws

from J. Sutton

Many countries bordering the Pacific contain rocks older than any preserved in the floor of that ocean where little material exceeds 200 million years in age and most is much younger. Yet the Pacific Ocean appears to have formed much further back in time and may have existed in the Precambrian, as long as 1,000 million years ago. Valuable clues to the early history of the Pacific can occasionally be picked up through study of old rocks preserved on land around its margin. Recent work by two members of the British Antarctic Survey on islands of the Scotia Arc has brought particularly interesting evidence to light. In this issue of *Nature* Smellie and Clarkson (page 701) describe new clues to possible conditions in Palaeozoic times along part of the south-east edge of the Pacific.

From studies around Scotia Arc, the 4,000 kilometre long ridge (in part submerged, in part exposed as groups of islands) which joins South America and Antarctica, they conclude that in Upper Palaeozoic times Pacific Ocean floor was being subducted and destroyed in that region.

They base their conclusions on their recent discoveries of the mineral glaucophane, a mineral found in metamorphic rocks which have been subjected to unusually high pressures and one which characterises many of the well-established subduction zones formed more recently in the history of the Pacific. Smellie and Clarkson found Palaeozoic glaucophane schists in the South Shetlands not far from the Antarctic Peninsula. Coupled with earlier discoveries of similar rocks among material dredged from the vicinity of the northern arm of the Scotia Arc, the new finds suggest the presence of a Palaeozoic glaucophane schist belt along which Pacific floor may have been subducted. Offset from part of this belt and roughly parallel to it a belt of rather different metamorphic rocks and intrusions has been identified on the Antarctic Peninsula through the work of many members of the British Antartcic Survey The nature of these rocks suggests a Palaeozoic belt formed by rather higher heat flow than that indicated by the glaucophane schist belt Smellie and Clarkson suggest that the two belts could be compared with the paired metamorphic belts which are a familiar feature of many younger terraines around the Pacific.

As a rule each pair comprises a metamorphic belt whose mineral make-up indicates a rather low heat flow parallel to a second belt further from the Pacific, formed by higher flux of heat The newly identified Antarctic belts follow this pattern and suggest there was an active Palaeozoic plate boundary between the south-east Pacific and the land masses of what is now Western Antarctica and southernmost South America.

That belt of subduction may well have preceded the opening of the South Atlantic and may have been active at a time when South America and Africa still formed a single block. Whether that block had separated from Antarctica at the period is uncertain The Palaeozoic rocks may have formed along the Pacific coast of Gondwanaland or have been part of an early island arc—a proto-Scotia Arc linking Antarctica with the South American–African continent.

Solving such problems entails careful examination of the older rocks preserved at intervals along the Scotia Arc. Recently, I. W. D. Dalziel has reviewed progress made between 1969 and 1975 on the Scotia Arc tectonics project mounted by Lamont (*Antarctic Journal of the United States*, 1975). There Dalziel summarises recent developments in the model for the evolution of the Scotia Arc earlier proposed by himself and Elliott, (*Nature*, **233**, 246–252; 1971). Dalziel and his colleague suggest that the Scotia Arc region was deformed in a Gondwanian orogeny in the early Mesozoic. From studies of Mesozoic sediment within the arc, notably on South Georgia and from within the South American continent they are able to suggest the Gondwanian orogeny resulted either from a collision of an island arc with the main part of Gondwanaland or from subduction beneath the margin of that super continent. Bit by bit the early history of the continental crust now widely dispensed along the Scotia Arc is emerging and the jigsaw of the present day fragments can be more confidently put back into place. ☐

Membrane-bound tubulin: fact or artefact?

from Mike Jacobs

The role of microtubules in the strategy of the cell is a very important one. The question of what functions they are actually involved in, is more difficult to answer and very much a subject of speculation. The main evidence for the participation of microtubules in such cellular activities as transport and secretion of cell constituents rests on inhibitor studies (for a recent review see Roberts *et al.*, *Prog. Biophys. molec. Biol.*, **28**, 371; 1974). These drugs can disaggregate microtubules by binding to tubulin, both in the cell and *in vitro*, and this is assumed to be their mode of action in inhibiting cellular processes. To prove this conclusively, however, is no easy matter. Colchicine, for example, inhibits the secretion of plasma proteins from the liver at the levels predicted by its binding constant to tubulin. Inhibition is accompanied by a dramatic accumulation of protein in the Golgi-derived secretory vesicles (Redman *et al.*, *J. Cell Biol.*, **66**, 42; 1975; Le Marchand *et al.*, *J. biol. Chem.*, **248**, 6862; 1973). Other obvious metabolic parameters are unaltered and colchicine analogues and drugs that do not bind to tubulin are ineffective.

The morphological evidence is not so good. Reports differ as to whether microtubules are depolymerised when secretion is inhibited. There are few microtubules in liver cells, and their orientation suggests no obvious role in secretion. Other studies of secretory cells often tell the same story; there is sparse anatomical evidence to support a mechanism by which microtubules transport 'packaged' molecules.

The finding of tubulin and colchicine

binding in the membraneous fraction of cells, may provide an alternative site for colchicine inhibition. Substantial amounts of tubulin are found in the post-synaptic functional lattice (Walters *et al*, *Nature,* **257,** 496; 1975) and in synaptic membranes and vesicles (Blitz *et al., Proc. natn. Acad. Sci. U.S.A* , **71,** 4144; 1974). There is little doubt that tubulin is present in these membrane preparations, as the protein was carefully characterised by SDS gel electrophoresis and peptide mapping. The main difficulty is to be sure that the soluble cytoplasmic tubulin is not trapped or adsorbed to the membrane during preparation.

Using thyroid and neuronal membranes, Bhattacharyya *et al.* (*J. biol. Chem.,* **250,** 7639; 1975) attempted to resolve this problem By adding radioactive tubulin the contamination of these membranes by cytoplasmic tubulin was estimated as 0.1%. Nevertheless, without knowing the relative specific activities of the membrane and soluble tubulin, it is still possible that this small contamination represents a significant proportion of the membrane protein Another problem is to consider what radioactive tubulin to add, as a label for soluble tubulin. 6S tubulin may not equilibrate with the ring form (36S) of tubulin or fragments of microtubules (Weingarten *et al., Proc. natn. Acad. Sci. U.S.A.,* **72,** 1858; 1975).

In these circumstances a better control may be to add radioactive microtubules. Another method of assaying the putative tubulin is by colchicine binding. In thyroid and neuronal membranes prepared by two different methods, colchicine binding activity is present far in excess of contaminating soluble tubulin. So the latter may not be such a problem. Colchicine binding to membranes by itself is not surprising since the molecule is so hydrophobic. What suggests that this binding site is indeed tubulin, is its respective affinities for colchicine and its analogues, and vinblastine, which are identical to those of soluble tubulin. Furthermore, the colchicine binding site can be solubilised with an enrichment in both its activity and in a protein co-migrating with cytoplasmic tubulin on SDS gel electrophoresis. The membrane colchicine site is more heat stable and has a higher optimum binding temperature than soluble tubulin, but reverts on solubilisation. Soluble tubulin in high sucrose shows a similar effect which is explained as due to preferential hydration preventing the protein unfolding (Lee *et al*, *Ann. N. Y. Acad. Sci*, **253,** 284; 1975). Such an explanation for the membrane-associated tubulin would imply a specific interaction between the two components. Further doubt as to the identity of the colchicine binding site on membrane should disappear when it can be definitely attributed to the solubilised membrane tubulin.

A role suggested for membrane-associated tubulin is as an anchor for cell surface receptors (Berlin *et al., Nature,* **247,** 45; 1975; Edelman *et al., Proc natn. acad. Sci. U.S A.,* **70,** 1442; 1973). Whether it does this in its unpolymerised form or as microtubules must be investigated. Then there are the other three fibrous protein, actin, myosin and spectrin, claiming association with membranes. How all these proteins function remains an exciting and intriguing question for the future.

QSO redshifts and the steady state cosmology

JOHN GRIBBIN (*Nature,* **257,** 540; 1975) has ably summarised the work by one of us (P.K.D.) which appeared recently (*Mon. Not. R. astr. Soc.,* **172,** 623; 1975) on the subject of the relationship between angular diameters and redshifts in Hoyle-Fowler type models of QSOs. Gribbin's ability as a mind-reader, however, must be seriously questioned when he writes in his preamble: " . . . although Das does not mention the steady state theory, it seems a reasonable assumption that any colleague of Narlikar's who discusses possible non-cosmological contributions to the redshifts of QSOs has the concept not too far towards the back of his mind."

While discounting that any such 'unholy' thought was at the back of our minds in our discussions of the Hoyle-Fowler objects (*Mon. Not. R. astr. Soc.,* **171,** 87; 1975), we would like to take this opportunity to point out that too much importance is being given by several astronomers and science writers (*New Scientist,* July 3, 4; 1975) to any connection between the steady state theory and the non-cosmological theory of QSO redshifts.

It is true that observations indicate that strong evolutionary effects are at work if the QSO redshifts are wholly cosmological in origin. It is also true that such evolutionary effects would be against the simple steady state hypothesis Thus a theory of non-cosmological origin of QSO redshifts is probably necessary for the survival of the steady state theory. But here the conection betwen the two ends.

For example, even if the QSOs turn out to have an appreciable non-cosmological component in their redshifts, this does not validate the steady state theory which still faces other observational hurdles like the microwave background. Nor does the disproof of a particular non-cosmological redshift hypothesis in a single QSO —for example, the gravitational redshift (Wampler, *et al., Astrophys. J.,* **198,** L49; 1975) in the case of 3C48 —particularly embarrass the steady state theory.

Indeed, regardless of any cosmological prejudices it is pertinent to ask: "Is the redshift of a QSO due to the expansion of the Universe alone?" A fair assessment of the present data does not permit an unequivocal affirmative answer to this question. Even the overall problem of the structure of a QSO still remains unsolved. Under these circumstances even theoreticians sympathetic to the steady state cosmology may work on models of non-cosmological redshifts for reasons not primarily concerned with defending that cosmology.

J. V. NARLIKAR
P. A. DAS

Lasers and the Lamb shift

from Peter Knight

THE investigation of atomic structure by conventional spectroscopic techniques (for example, excitation using electron-impact in a discharge tube and resolution of the emitted radiation with interferometers) is severely limited by Doppler broadening and consequently relies heavily on a precise understanding of the lineshape of the emitted radiation. To see the subtle effects of current interest within Doppler broadened spectral lines, with widths typically of the order of GHz, the spectroscopist is forced to the limits of available technology. This is especially true of that most "fundamental" of atoms, the hydrogen atom, which, being the lightest atom suffers most from Doppler broadening. The arrival of the tunable laser in atomic physics has, however, totally revolutionised optical spectroscopy. This long-awaited revolution is perhaps best characterised by a series of beautiful experiments on atomic hydrogen using dye laser excitation performed by T. H. Hänsch and coworkers at Stanford. In a recent paper (*Phys. Rev. Lett.,* **35,** 1262–1266; 1975) Hänsch, Lee and Wallenstein report measurements of the isotope shift of the 1s–2s transition in atomic hydrogen and deuterium, and the Lamb shift of the 1s ground state of hydrogen, both very small effects compared with the (optical or ultraviolet) transition frequency. As a demonstration of the power of their new technique, they report improvements of several orders of magnitude over previous measurements of this isotope shift.

The key feature is the ability of the laser spectroscopist to overcome the Doppler broadening of spectral lines. The monochromaticity of the laser allows selective excitation of atomic excited states. At the same time the laser intensity is high enough for non-linear absorption effects to be exploited: one of these is saturated absorption. A laser beam, with a linewidth much less than the Doppler width of the spectral line of interest, interacts not with all atoms in the absorption cell, but with a set of atoms with resonance frequencies (and therefore velocities) in a narrow range around the laser frequency. An intense beam of laser radiation will excite this set of atoms: it saturates the gas medium and burns a "hole" in the velocity distribution of ground-state atoms. In saturated absorption spectroscopy the output of a laser is split into a weak probe beam and a stronger, chopped saturating beam of the same frequency which are sent in nearly opposite directions through the absorbing gas. If the laser frequency is close to an atomic resonance frequency, the saturating beam bleaches a path for the probe and decreases its absorption, but only for that group of atoms with effectively zero velocity along the laser beam-path. Moving atoms see the two beams as of different frequencies, and no such bleaching occurs. In this way the Doppler effect is almost eliminated. The saturation spectrum is obtained by varying the laser frequency and recording the change in the weak probe beam due to the inhomogeneous saturation created by the strong bleach beam. The theoretical limit to the linewidth is then governed by the natural lifetime of the excited state.

A second technique to eliminate Doppler broadening is to use two-photon absorption. This time the laser is split into two counter-propagating beams of equal intensity. This may be achieved merely with a mirror at one end of the cell to reflect the incident light back down the cell. If the laser frequency ω is approximately one-half of an atomic resonance frequency ω_0, an atom can be excited by the absorption of two photons, one from each oppositely travelling wave. An atom of laboratory velocity $\mathbf{v}$ sees, in its rest frame, two beams of frequency $\omega(1 - v_x/c)$ and $\omega(1 + v_x/c)$. At resonance, $\omega_0 = \omega(1 - v_x/c) + \omega(1 + v_x/c) = 2\omega$, independent of the velocity of the atom. Therefore Doppler broadening is eliminated if the atom absorbs two photons propagating in opposite directions, and again the natural lifetime of the excited state is the limiting factor in the linewidth. The two-photon absorption signal has contributions from all atoms in the cell, irrespective of their velocities. This technique also makes possible Doppler-free spectroscopy of s→s or s→d transitions, not possible by one-photon saturated absorption.

A hundred years ago

THE ravages of the Phylloxera among the vines have caused many attempts to be made to discover a new kind of beverage which might take the place of the grape. The Marquis de Villeneuve reports that in China a *pseudo* wine called *Tsien-ia* is much used, which is concocted from a preparation of four plants, common in that country, and mixed together in certain proportions. The plants are dried and powdered, and made into a paste, which is sold in the form of balls or squares at the rate of about 3*d.* a pound. One square or ball will make several pints of a fermented liquor, pleasant to the taste and much resembling wine, which is much sought after by Europeans and others living in China.

from *Nature*, 13, December 30, 175; 1875.

Hänsch and his co-workers have simultaneously used two-photon absorption and saturated absorption Doppler-free spectroscopy in their hydrogen work.

A pulsed dye laser output at ~ 4,860 Å in 10 ns pulses of linewidth 120 MHz, is split into two beams. Noting that the crude Bohr theory predicts that the $n = 1$–2 interval is four times larger than the $n = 2$–4 interval, they contrive to frequency-double one beam (so that its wavelength is now ~ 2,430 Å) and excite the $1S_{1/2}$–$2S_{1/2}$ Lyman-α transition at 1,215 Å in a cell of hydrogen or deuterium atoms, by two-photon absorption. Since an isolated atom in the $2S_{1/2}$ state has a lifetime of ~ 0.14 s this transition could potentially be measured to very great accuracy. At the same time the second beam is used in a saturated absorption spectrometer to measure the $2P_{3/2}$–$4D_{5/2}$ transition interval at 4,860 Å in a separate cell. By varying the laser frequency, therefore, both transitions can be simultaneously recorded. The measurements are not made in the vacuum ultraviolet, liquid air cooling of the discharge and the use of large gratings is avoided, yet great accuracy is possible.

If the Bohr theory were correct, the two observed transitions would occur at exactly the same dye-laser frequency. The fact that they are displaced is due to quantum electrodynamic effects (principally the Lamb shift of the various levels), relativistic effects, and partly to nuclear structure effects. The measured displacement between the two transitions gives directly information about the size of these effects without the need for absolute wavelength calibrations or an ultra-precise value of the Rydberg constant. By comparing the experimental and theoretical line separations, Hänsch *et al.* deduce a 1s Lamb shift of 8.20 ± 0.10 GHz for H, and 8.25 ± 0.11 GHz for D, which agree within their error limits with the theoretical values. At the same time they obtain a 1s–2s isotope shift in atomic hydrogen and deuterium of 670.933 ± 0.056 GHz, in fair agreement with the theoretical value, but representing an improvement of several orders of magnitude over previous (traditional pre-laser) measurements. To appreciate the leap in accuracy made possible by Doppler-free laser techniques, this measured isotope shift should be compared with the hydrogen Lyman-α line Doppler width of ~ 6 GHz.

To strike fear into the hearts of theoreticians, they point out that much more accurate experiments are possible using two-photon absorption in the 1s–2s transition, and two-photon absorption from a GaAs diode laser, whose second harmonic is locked to the dye-laser frequency, driving a 2s–4s transition. Given the lifetimes of the various participating levels, they feel the ultimate accuracy of such proposed optical experiments could exceed recent high-precision radiofrequency measurements of the 2s Lamb shift, and even that of present day theory of the Lamb shifts.

These elegant experiments of Hansch and coworkers emphasise the part that the tunable laser has played and will play in precision spectroscopy, a role that it has only recently assumed yet which has already produced a qualitative change in the direction of atomic physics. □

Heat flow on different scales

from Peter J. Smith

UP to the late 1960s, heat flow studies were generally regarded as interesting and potentially important but of little immediate use. Their underlying importance was acknowledged because heat plays a part in almost every conceivable terrestrial process, but the promise they held out to illuminate the nature of such processes was difficult to realise. Part of the problem was that data coverage was poor and there were large areas of the world with few or no heat flow observations Such areas still exist, unfortunately, but they present fewer difficulties than before as emphasis has now shifted somewhat from global analysis towards the application of heat flow data to component parts of the global tectonic system, whether they be oceanic ridges, subduction zones or even whole oceans.

A good example of this trend towards smaller-scale phenomena is provided by R. N. Anderson (*J. geophys. Res* , **80**, 4043, 1975) who has used heat flow observations to elucidate the nature of the Mariana trough. The interpretation of the marginal basins which lie behind island arcs in the western Pacific has long been a matter of debate. The suggestion that such basins are extensional features was made as long ago as 1954 and has been repeated many times since, although it was left to Karig (*J. geophys. Res.*, **75**, 239; 1970 and *Geol. Soc. Amer. Bull.*, **82**, 323; 1971) to provide the detailed geological evidence to support it. Karig showed that marginal basins are bounded by steep normal fault zones, that the bounding arcs are covered with thick sediment whereas the basin interiors are almost sediment free, and that in some cases within-basin fault block morphology can be traced onshore as a graben system

These and other results led Karig to conclude that marginal basins in general and the Mariana trough in particular were formed by recent intrusion and crustal extension—a view now widely, though perhaps not universally, held. But even if this is accepted, it still leaves open the question of just how the extension occurs. Karig himself proposed extension by dyke intrusion along the central axial high of the Mariana trough, although he also produced evidence suggesting that the rate of extension decreases towards the edges of the basin. In a sense, this is an intermediate view between that of Sclater (*J. geophys. Res.*, **77**, 5705; 1972), who proposed a mechanism akin to seafloor spreading but with the production of a thinner than normal crust, and that of Hasebe *et al.* (*Tectonophysics*, **10**, 335; 1970), who suggested a slow diapiric rise of magma ('incipient intrusion') throughout the basin.

There are no regular magnetic anomalies over the Mariana trough of the type usually taken to support seafloor spreading; but this is not conclusive evidence against spreading because any anomalies would probably be disrupted by the rough topography and many transverse fracture zones. It is at this point therefore that heat flow should be able to settle the matter, for the different models proposed give rise to different heat flow regimes. Unfortunately, most heat flow values obtained from the Mariana trough so far have been abnormally low, thereby appearing to support none of the models and creating what Watanabe *et al.* (*Tectonophysics*, **10**, 205; 1970) have called an "anomalous, difficult situation".

But 21 heat flow measurements now reported by Anderson tell a different story. With one exception, both new and old values on the flanks of the axial high are indeed low—lower than the 1 1 h.f.u. mean for the oldest parts of ocean basins and in one case zero—irrespective of whether the stations were in sedimentary ponds, on flat sedimentary blankets or on local topographic features. On the axial high itself, however, high heat flow was observed—in one case 8.45 h.f.u. In short, with sufficient data coverage the distribution of heat flow across the Mariana trough broadly resembles that across an oceanic ridge Anderson's conclusion, therefore, is that the trough is being produced by seafloor spreading with a narrow zone of magnetic intrusion centred on the axial high.

The wide range of environments in which heat flow over the flanks of the axial high was universally low led Anderson to attribute the low values to hydrothermal circulation. Fluid circulation also plays an important part in Albarede's study (*Earth planet. Sci. Lett* , **27**, 73; 1975) of the linear relationship between heat flow (q) and surface heat production (A) in continental plutonic rocks, discovered by Birch *et al.* (in *Studies of Appalachian Geology*, Interscience, 1968). The simplest interpretation of this relationship ($q=a+bA$, where a and b are constants over large areas known as 'heat flow provinces') is that radioactivity is constant to a depth b and zero below. As Lachenbruch (*J. geophys. Res.*, **73**, 6977, 1968) pointed out, however, this interpretation is inconsistent with differential erosion within a heat flow province; instead, radioactivity must decrease exponentially with depth.

But how does this exponential dependence arise physically? Various suggestions have been put forward ranging from the effects of fractional crystallisation of cooling magmas to equilibrium established by gravitational forces There are objections to all of them; and so Albarede has developed a new model, based on the known mobility of the radioactive elements U and Th, in which a redistribution of radioactive elements is achieved by migration of water and other fluids through the magma during crystallisation and cooling.

Meanwhile, the global picture is not being ignored completely, for Chapman and Pollack (*Earth planet Sci. Lett.*, **28**, 23; 1975) have carried out an important new analysis of all heat flow data which finally attempts to come to terms with the problem of poor coverage. New spherical harmonic analyses of world heat flow observations have appeared every few years since 1963 as the data set has grown, but they have all suffered from distortion caused by lack of measurements in certain critical areas (notably Africa). To overcome this difficulty in the most satisfactory way would require thousands of new measurements—which are simply not going to be made, at least within the foreseeable future.

Chapman and Pollack have therefore 'manufactured' the missing results using predictors based on known correlations: for continents, the observed correlation of heat flow with the age of the last tectono-thermal event, and for oceans, the observed relationship between heat flow and the age of the ocean floor. The resulting 12-degree spherical harmonic analysis (and its pictorial representation) is probably not far off that likely to arise from completely genuine data. It yields a world mean heat flow of 59 mW m^{-2} (1.41 h.f.u.). □

The nucleolus on the Black Sea

from a Correspondent

The Fourth Nucleolar Workshop was held at Varna, Bulgaria, on September 14–19, sponsored by the European Cell Biology Organization and the Bulgarian Academy of Sciences. Further information can be obtained from the organiser, Professor A. A. Hadjiolov, Institute of Biochemistry, Sofia.

A MULTIDISCIPLINARY approach and a warm and friendly atmosphere contributed to make an exciting and successful meeting. During discussions of the nucleosome structure of chromatin P. Oudet (University of Strasbourg) reported results showing that they are 128 Å spheres of 194-200 nucleotide pairs and the four histones $F2a_1$, $F2a_2$, F2b and F3, while R. Tsanev (Institute of Biochemistry, Sofia) gave evidence for two internal supercoils of DNA per nucleosome wrapped around a protein core. Amongst the many questions yet to be elucidated is whether nucleosomes are present in actively transcribing chromatin. They have not been seen in active rRNA genes nor in lampbrush loops and they seem to characterise inactive chromatin.

Contributions on nucleolar fine structure brought about some modifications to the classical morphological model of the nucleolus. Evidence was presented by G. Goessens (University of Liege) that the fibrillar centres contain protein and some DNA, are labelled by ^{3}H-uridine pulses and are always associated with the dense fibrillar RNP components. In some cell types, the fibrillar centre can be followed during mitosis, it is also present in new-born nucleoli of heterokaryon cells (M Bouteille, Paris).

Variation in nucleolar size and distribution of nucleolar components can reflect changes in activity. The formation of micronucleoli in ageing cells, characterised by degranulation and absence of condensed chromatin, seems related to cessation of RNA synthesis (K. Smetana, University of Prague). In contrast, small nucleolar fragments caused by α-amanitin retain the granular component and remain active in RNA synthesis (R. Simard, University of Montreal) The existence of a true nucleolus was demonstrated also in yeast cells by B. Stevens (University of Paris XI).

Evidence for a nucleolar compensation mechanism was given by V. Sakharov (Institute of Chemical Physics, Moscow) following ultraviolet microbeam irradiation of one of two interphase nucleoli. Cinematography showed an increase in volume of the unirradiated nucleolus simultaneous with a shrinkage of the damaged one.

The beautiful demonstration by the group of W. Franke (Cancer Research Centre, Heidelberg) of the fine anatomy of rRNA transcriptional units using the O. Miller spreading technique brought several new results. The start of the "Christmas Tree" may not correspond exactly to the initiation point since "prelude" transcripts are often seen and occasional long RNP fibrils may be interspersed in the initiation region, indicating that transcription and processing may go on together. The view that the primary transcript is larger than the first stable product and that the spacer is transcribed but immediately cleaved is supported by examples of long transcription units with almost no spacer and by 5-fluorouracil treatment where the spacer is eliminated and a larger than 40S RNA molecule is found in *Xenopus*. Regulation of transcription seems to depend on the rate of initiation. The concepts of heterogeneity and individuality of rRNA genes within one species and even within one nucleolus were subjects of some discussion.

Mapping of genes by restriction enzymes produced two important contributions. The five histone genes were shown by W. Schaffner (University of Zurich) to alternate with each other instead of being clustered in five separate groups J. Engberg (University of Copenhagen) demonstrated an unusual arrangement of the rRNA genes in *Tetrahymena* as a palindrome with two gene copies per intact 12×10^6 linear molecule.

Conservation of some rRNA sequences during evolution was demonstrated by S. Gerbi (Brown University) based on competition hybridisation and comparative fingerprint analysis and by M. S. Khan (University of Glasgow) and J. Klootwijk (Free University, Amsterdam) who found conservation occurred around methylation sites.

The three nuclear RNA polymerases can be manipulated and characterised by their different sensitivities to α-amanitin and heat inactivation. The yeast polymerase A studied by J. Retel (Free University, Amsterdam) is active in rRNA transcription *in vitro* with a preference for a high physical integrity of the DNA template and a low enzyme/DNA ratio. Using a technique of subnuclear fractionation J. Tata (National Institute for Medical Research, Mill Hill) emphasised the compartmentation of events in the nucleus. Polymerase B is found exclusively in the nuclear sap and euchromatin-rich fractions and most processing and polyadenylation occurs close to the site of synthesis

Maturation of rRNA precursors may involve the simultaneous occurrence of more than one pathway (M. Dabeva, Institute of Biochemistry, Sofia) and the two rRNA species mature at different rates and sites In *Chironomus*, the cytoplasmic distribution of newly-labelled 28S rRNA follows a gradient up to 6 days (U Lonn, Karolinska Institute, Stockholm).

Research on extranucleolar sites of RNA synthesis and processing has received much impetus from collaborative efforts by J. P. Zalta (University of Toulouse) and E. Puvion and G. Moyne (Institut du Cancer, Villejuif) combining biochemical fractionation and electron microscopy. Newly-synthesised RNA can be located in perichromatin fibrils at the periphery of condensed chromatin; the labelling profile of this product corresponds to HnRNA.

A keyword of the meeting was post-transcriptional regulation S. Penman (MIT, Boston) showed that growing cells process more of their pre-mRNA into cytoplasmic poly(A)-mRNA than do resting cells. Evidence was presented (K. Scherrer, I.B.M., Paris; E. Egyhazi, Karolinska Institute, Stockholm; E. Serfling, Genetics Institute, Gatersleben) that pre-mRNA genes may be continuously transcribed in all cells but that control resides in the efficiency of processing into mature mRNA.

The next Nucleolar Workshop is planned for 1977 in Salamanca.

A most Friendly meeting

THE article regarding "a most Friendly meeting" (*Nature*, **257**, 741; 1975) was flattering, but unfortunately misleading, in that it described as being completed experiments which have not yet been accomplished. Specifically, heterokaryons formed by the fusion of DMSO-induced tetraploid mouse erythroleukaemia cells with diploid human amniotic fibroblasts were said to have synthesised human globin. Unfortunately, these heterokaryons so far synthesise only mouse globin. It is possible that the authors were referring to experiments reported by one of us (Deisseroth A., *et al.*, *Proc. natn Acad. Sci U.S.A.*, **72**, 2682; 1975), in which continuously proliferating hybrid cells were generated by the fusion of human marrow erythroid cells with diploid mouse erythroleukaemia cells. These hybrids, when induced with DMSO, contain both human and mouse globin messenger RNA.

We do not want readers to think that human fibroblasts have been as yet induced to synthesise hemoglobin; these experiments are in progress.

BLANCHE ALTER
ALBERT DEISSEROTH

Astronomy from the Space Shuttle

from John Gribbin

A discussion meeting of the Royal Astronomical Society and the Institute of Physics—Uses of the Space Shuttle for Astronomy and Geophysics—was held on December 12 in London.

THE meeting highlighted the very different approach to scientific experiments in space necessitated by the advent of the shuttle. Previously, would-be experimenters have to some extent operated on the basis of envisaging a worthwhile experiment and then looking for a suitable way to get it off the ground; now, the American and European space agencies (NASA and ESA) are providing a standard launch vehicle and modular space laboratory, and it is up to the experimenters to make the best use of the facility provided

J. Burger (ESA) set the scene with a background talk describing the work-horse vehicle for low orbit work in the 1980s. The supposed prime reason for the development of the Shuttle is the economy of a re-usable facility There has been some debate about just how economical the Shuttle is likely to be, but some scientific good may emerge from the political debate since in order to be cost effective the vehicle must certainly be flown as frequently as possible. The advantages of the Shuttle/Spacelab in providing a facility for repeated experiments, retrieval and repair of satellites and reducing the lead time from idea to orbiting experiment are in large measure counterbalanced by the need to work within a well-defined framework and the restriction of flights to between 7 and 30 days in orbit. Although Burger did not say so, the real situation from the scientists' point of view seems to be that this is the best Shuttle we've got, and with no other major launch facility on offer we had better make the most of it.

The basic facility is a Spacelab built up from 4 m diameter modules with a shirtsleeve environment and pallets open to space. Depending on the exact configuration, the mass carried is in the range 5,500-9,100 kg, with a floor loading of about 500 kg m^2 very similar to that in buildings; the volume of the closed modules may be between 5 and 8 m^3, and the length of open pallet may be as little as 3 m or as much as 15 m Up to four specialist experiment operators will be carried on Spacelab flights, and they will have available 4-5 kW of power with a peak short term facility of 9-10 kW and a total of 400-600 kW h at 28 ± 4 V. The Spacelab/Shuttle will not use solar cells, and depends on stored power. An onboard computer (64 k) will provide some data processing in orbit, and the stability of this space platform will give arc second pointing accuracy for payloads of around 2,000 kg.

Potential users of the facility seemed, on the evidence of this meeting, to divide between those (such as solar observers) who felt that 7-30 days in space is severely limiting and those (such as the rocket X-ray experimenters) who seemed almost overwhelmed at the prospect of such lengthy observations. This perhaps emphasises that Spacelab is unlikely to be ideal for anybody but will probably offer something for everybody. The infrared observers, represented by R. E. Jennings (University College, London), are among those to whom even 7 d observing is a long time (compared with balloon flights of a few hours) and plans are going ahead for two major telescopes, a 2-3 m instrument operating at ambient temperature with high spectral and spatial resolution, being studied by ESA, and a cryogenically cooled instrument with high sensitivity but limited size, being studied by NASA. One major compromise in Jennings' eyes is the presence of people on the Shuttle and the need to test life support and other systems before venturing on long missions. He would like to see pallet-only missions lasting 30 days as soon as possible, without "people hopping about" inside the vehicle.

R Griffiths (University of Leicester) pointed out that the high energy region of the spectrum cannot be studied from ground-based observatories at all, and that the observation time provided by Spacelab will be at least 10^4 times more per flight than that provided by rockets. Simply flying tried and tested rocket type X-ray detectors on a Spacelab pallet is certain to produce an enormous increase in knowledge about the Universe at these wavelengths, and there is likely to be a Small Instrument Package System (SIPS) to provide mounting and accurate pointing for pairs of small experiments which can thus almost ride piggyback on a major Spacelab flight. But there is also the potential with Spacelab for a further quantum jump in observing techniques at X-ray frequencies, including development of a planned 60 cm telescope (3 m focal length) providing the facility for a variety of focal plane observations. Gamma ray astronomy seems at last to be producing something more than negative observations and upper limits, and will obviously benefit from the opportunities of the Shuttle; cosmic ray studies could move into new areas with searches for anti-nuclei and perhaps determination of isotope abundances, and one possibility for Spacelab is a complete high energy payload of X, gamma and cosmic ray equipment on one pallet.

This type of coordinated package also provides the major advantage of Spacelab to the solar physicist. A. H. Gabriel (Appleton Laboratory, Slough) stressed the need for coordinated observations of solar phenomena at a variety of wavelengths, giving a simultaneous picture of events at different temperatures and different heights in the solar atmosphere. The high resolution available above the atmosphere is equally important, and to round things off the first flight of Spacelab in 1980 should give time to have everything running smoothly in time for observations of the next solar maximum. But even 30 days is, of course, a short time for observations of many solar phenomena.

P. W. Hill (University of St Andrews) received the roughest ride, both in the limited question time and in private discussions afterwards, for his efforts to justify Spacelab as an observing platform in the visible part of the spectrum. It was generally agreed that a 1 m telescope in orbit could produce results comparable to the 200 inch on the ground—but that it would probably cost at least as much as a new 200 inch. If the Shuttle was to be an optical astronomy facility alone, it would be a waste of time and money; an hour's observing at a major ground based observatory costs about £50, and Hill quoted an estimate that an hour's observing from the Shuttle will cost £50,000. Only in the far ultraviolet do the advantages of the Shuttle really become valuable for conventional-type optical astronomy, and an orbiting ultraviolet Schmidt camera to produce a sky survey at these wavelengths could be particularly valuable.

The value of the Shuttle for atmospheric, magnetospheric and plasma physics (the AMPS package) seems to be largely the opportunity to 'kick' the near-Earth environment and see what happens. L. Thomas (Appleton Laboratory) foresaw that by the 1980s we will have done the basic exploration of the magnetosphere through planned satellites, and will be entering the phase of testing hypotheses and looking for cause and effect relationships. Spacelab is certainly an appropriate platform for such experiments, and as well as the opportunity to study the magnetosphere by firing lasers, masers and electron streams into it, there is ample scope to monitor the upper atmosphere by observations across a chord of the tenuous upper layers providing long path lengths and giving data on low concentration constituents. Perhaps Spacelab will finally resolve the ozone/SST/freon.

Surprisingly, however, the most immediate and obvious astronomical benefits from Spacelab — and those which are certain to catch the public eye—will be the first photographs of the planets from a modest orbiting telescope. A 1 m telescope in orbit, said G. Hunt (Meteorological Office) will produce pictures of Uranus as good as the best ground-based pictures of Jupiter, pictures of Jupiter with resolution of 250 km or better equivalent to those from the Pioneer spacecraft, and comparably good pictures of the other planets. The particular significance of this is that the facility will provide many pictures over a period of years, whereas the spaceprobes provide only one-off series of snapshots. The advantages in the study of planetary atmospheres—the rapid rotation of Venus' atmosphere, the dust storms on Mars, the disturbances and spots on Jupiter—are obvious. But if the series of planetary space probes from NASA are remembered by most people simply for their photographs, it seems inevitable that the public image of Spacelab will be a platform for viewing the planets. ☐

review article

Quantitative variation and gene number

James N. Thompson, Jr*

Gene number estimates from fine structure analysis seem to be incompatible with the number of genes often presumed to affect quantitative characters. This paradox can be resolved by reconsidering the available evidence about the nature of polygenes.

MUCH of the discussion about the number of genes in eukaryotic genomes has centred on the discrepancy between the probable gene number, based on mutation saturation rates and fine structure analyses, and the apparent coding potential of the DNA[1-5]. The haploid *Drosophila* genome, for example, contains enough DNA to code for over 100,000 genes. Recent studies, however, tend to support the idea that the actual number of genes is only about 5,000, approaching a 1 : 1 correspondence between functional units and bands or interbands in polytene chromosomes. Thus, the actual number of genes appears to be 1 or 2 orders of magnitude less than the number which could be coded for by the available DNA. Such a relationship clearly has important implications for chromosome organisation and for the control of development[6,7].

But quantitative genetics often seems to make quite different assumptions about gene number. Many characters, such as human height or tail length in mice, are considered "polygenic"; that is, genes which affect them are believed usually to have effects that are small relative to those of other sources of variation. A normal phenotypic distribution for such a character is often interpreted as showing the segregation of a large number of genes. In addition, the fact that the expression of hundreds of mutant alleles can be modified by selection is believed to indicate that these selection responses are often due to changes in the linkage of a large number of polygenes.

This seems to present a paradox. Deletion analyses and studies of lethal complementation groups suggest that there is a comparatively limited number of genes affecting development, while studies of quantitative characters seem to uncover a vast number of genes having morphological effects.

But this paradox is really traceable to basic misunderstandings about the nature of polygenic variation. "Polygene" is a nonspecific term used to describe what is probably a very heterogeneous set of gene effects. It was originally defined in the context of biometrical analysis[8]. One of the early, and quite necessary, assumptions which biometrical models made about genetic systems was that quantitative variation was produced by many genes having small, similar effects Unfortunately, some have since come to think of this description as an established fact rather than as a simplifying assumption.

It is appropriate, therefore, to ask whether one must necessarily conclude from a normal phenotypic distribution that a given character is affected by the segregation of a large number of polygenes. The answer is "no", a large variance can be caused by the segregation of only a few genes just as it can be caused by the segregation of a large number. The theoretical distribution which underlies the sample shown in Fig. 1*a* is produced by the segregation of only three genes. One of these has three times, and one has twice, the effect of the third gene. The distribution approaches a normal distribution, even though only two loci account for more than 90% of the variance. When the effects of environmental variation are taken into account (Fig. 1*b*) even segregation at a single locus can produce a continuous distribution of phenotypes. Clearly, there is no justification for automatically assuming that a continuous distribution reflects the segregation of a large number of polygenes.

Although experimental analysis of quantitative characters is usually more complex, some bristle and venation characters in *Drosophila melanogaster* have confirmed that a relatively small number of genes can account for the observed variance and response to selection.

Many major mutants affect the formation of wing veins

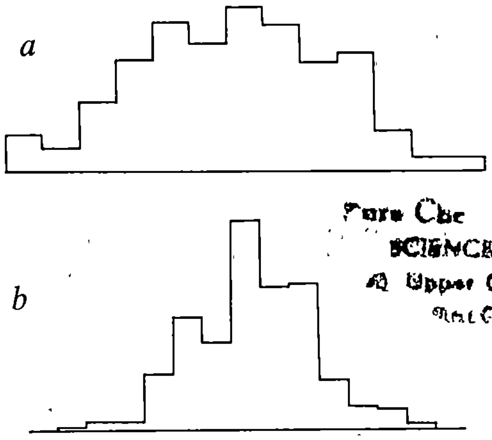


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Fig. 1 *a*, Random samples of 150 individuals taken from a theoretical distribution of only three segregating loci. The effects of the environment have not been considered in this example. Superimposing environmental variation on this distribution of phenotypes, as is typical of quantitative characters, would blur phenotypic classes even more. *b*, Random sample from an F_2 in which only one locus is segregating. The effects of environmental variation have blurred the classes to produce a more nearly continuous phenotypic distribution. In this example, heritability is about 50%. Clearly, there is no justification for automatically assuming that a continuous distribution reflects the segregation of a large number of polygenes.

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in *Drosophila*. Not surprisingly, the expression of vein length in these mutants is, in turn, affected by polygenes, and vein length responds readily to artificial selection. Studies of these vein length polygenes show several things. One striking observation is that all tested natural populations seem to be polymorphic for polygenes affecting vein length—all samples of wild populations are segregating for alleles which either lengthen or shorten the veins of mutant flies by a small amount, although these polygenes do not have any recognisable phenotype in the wild flies in which they are found.

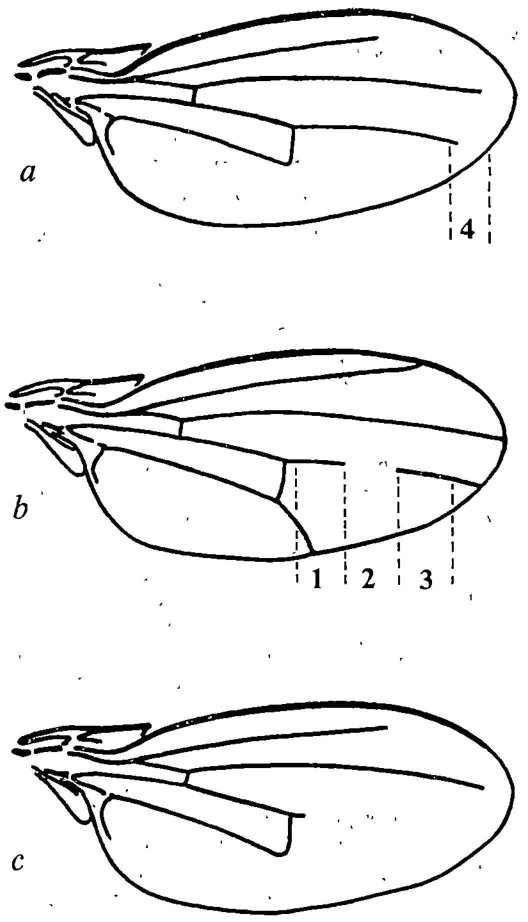

Fig. 2 Wings of *Drosophila melanogaster*, showing regions of the fourth longitudinal (L4) vein affected by individual polygenes. The difference in expression of *ve* Long (*a*) and *ve* Short (*c*) selection lines can be accounted for by only two or three loci. One of these (located on the right arm of chromosome II, ref. 23) eliminated vein from the middle of the L4 vein (*b*, region 2). The other polygenic loci influence the expression of the second chromosome polygene by enlarging the gap formed in the middle of the L4 (regions 1 and 3). The same gene or genes may act on both regions 1 and 3. The *ve* mutant itself does not form vein at the tip (region 4), even in Long selection lines. Thus, the *ve* Short phenotype (*c*) is simply the summation of the background effects (mid-vein) and the *ve* phenotype (distal-vein), leaving the base of the L4, which is not affected by either set of genes, intact.

One might interpret this observation as showing that a large number of polygenes affecting vein formation exist in nature. On closer examination we find that this is not necessarily true. By locating regions in a chromosome which have an effect on the character, one can isolate individual polygenic loci[9]. Even though a polygenic locus could be a complex of two or more closely-linked loci, this technique still gives us a reasonably clear idea of the number of factors which account for the majority of the genetic variance. In the case of polygenes affecting vein length in the mutant *veinlet* (Fig. 2), only two or perhaps three loci accounted for most of the difference between a line selected for longer and one selected for shorter L4 veins.

The same general conclusion emerged from studies of polygenes affecting sternopleural bristle number in *Drosophila melanogaster*. Thoday and his colleagues[10–13] found that only five loci accounted for more than 85% of the difference between a high line having a mean of about 40 bristles a fly and a control line having about 20 bristles a fly. (Biometrical, rather than genetic, estimates suggest that approximately 100 polygenes affect a similar character—abdominal bristle number[14].)

In this context, however, it must be remembered that one can only study genes affecting the variance of a character in a population sample. Although a given polygene can appear in more than one population[15,16], each population may carry its own polygenic combinations. There may also be polygenes which have extremely small effects. These can not generally be examined by standard polygene location techniques, but by the same token they will have only limited importance for the determination of the variance. Many of these may be pleiotropic effects of other loci, though there is little evidence on this point. In addition, there may be undetected homozygous alleles in the sample being measured, since one can only detect segregations contributing to the variance. All of these situations will increase the total estimate of polygene number. But even with these reservations, there is no compelling reason to think that the total number of loci affecting any particular quantitative character is exceptionally large.

The evidence that a relatively small number of genes can, in principle, account for the genetic variation of various characters solves only part of the problem. How does one reconcile the fact that the sum of all polygenes affecting all characters still produces an estimate which is inconsistent with those of Judd[1,3], Hochman[2], and others? In addition, is it possible to explain complex characters, such as body weight, in which it appears that large numbers of polygenes are actually involved? These two questions can best be answered by considering the possible functions of individual polygenic loci.

Again, venation and bristle characters in *Drosophila* are model systems. If the polygenes affecting these characters are specific modifiers of a particular locus (suppressors or enhancers affecting the control of the mutant locus—see, for example, ref. 17) the total number of polygenes in nature must be much larger than the number of mutants having morphological effects. If, on the other hand, polygenes influence some step in a particular developmental process and only indirectly modify the expression of a mutation in the same process, the minimum polygene estimate can be greatly reduced because one polygene could contribute to the variation in a number of different mutant phenotypes In the first situation, polygenes might be a special class of control genes; in the second situation, they may simply be minor variations at familiar loci. Although a few mutant-specific polygenes have been reported[18–20], recent studies of the modifiers of vein mutants support the second hypothesis, that, in general, polygenes seem to act primarily on some developmental step and only indirectly modify mutant expression[21–23].

Some of the evidence for this conclusion is summarised in Table 1. Six different vein mutants[24] were selected for longer or for shorter vein length (*veinlet, radius incompletus, short vein* and *cubitus interruptus*) or for extra vein fragments (*plexus* and *net*). Selection accumulates in each line the polygenes which have the relevant effect on the phenotype. By comparing the contribution of individual chromosomes with a standard, the overall phenotypic effects of the polygenes on each chromosome can be measured. Recombination studies of some of these lines show that the majority of the effect of each chromosome is due to only

Table 1 Summary of chromosome substitutions of selected polygenes from one mutant background in to another

Vein affected by polygenes	Chromosome substituted*	First mutant (source)†	Second mutant	Effect in first mutant	Effect in second mutant
2	II	*ve* L-2	*ri*	+	+
2	II	*ve* L-3	*ri*	+	+
2	II	*ve* S-2	*ri*	+	+
2	II	*ri* L	*ve*	+	+
2	III	*tg^C* S	*shv*	—	—
3	II	*ri* L	*ve*	None	None
3	II	*ri* S	*ve*	None	None
4	II	*ve* S-2	*ci*	—	—
4	II	*ve* S-4	*ci*	—	—
4	II	*ri* L	*ci*	None	+
4	II	*ri* L	*ve*	None	None
4	III	*shv* L	*ci*	+	+
4	III	*shv* S	*ci*	—	—
4	III	*ve* S-4	*ci*	—	—
4	III	*ve* S-4	*shv*	—	—
4	III	*px* L	*shv*	+	+
4	III	*px* S	*shv*	—	—
4	III	*net* L	*shv*	+	+
4	III	*net* S	*shv*	—	—

These substitutions confirm that at least a proportion of the polygenes affecting vein length have the same influence on the phenotypes of non-homologous mutants.†

* Chromosome extracted from the selection line (without carrying a venation mutant) and substituted in to a control line of the second mutant.

† The original selection line. L, longer veins or extra vein fragments; S, shorter veins or fewer vein fragments; numbers refer to tests of parallel selection lines of the same mutant.

‡ Mutant symbols: tg^C (telegraph of Carlson) and *ri* (*radius incompletus*) shorten vein 2; *ci* (*cubitus interruptus*) shortens vein 4; *ve* (*veinlet*) and *shv* (*short vein*) shorten all veins; *net* (*net*) and *px* (*plexus*) have fragments of extra venation.

one or two loci. This is consistent with the earlier studies on polygene number.

The next step is to test the specificity of the polygenes on each chromosome. Using marked balancers, chromosomes from one mutant line can be substituted for a control chromosome in the genome of a different vein mutant. It is clear from the substitutions summarised in Table 1 that the vein length polygenes on these chromosomes act in a general way on vein formation insofar as they have qualitatively similar effects upon the phenotypes of different mutants. Those which lengthen the second longitudinal vein in *veinlet*, for example, also lengthen the second longitudinal vein in *radius incompletus*. Those which decrease the amount of extra venation in *plexus* also decrease the amount of vein in *short vein*. With one minor exception, all of these substitutions support the hypothesis that a proportion of the polygenes are acting in a general way on some aspect of vein formation and only indirectly influencing the expression of related mutations. This, therefore, reduces the number of polygenic loci which one must postulate to explain the vast amounts of variations observed in mutant expression. I am confident that many such examples could be found.

Another point which emerged from the analysis of vein expression polygenes was that they were often specific to one region of the wing. One polygenic locus would affect the formation of one vein only. This was most clearly shown by selection in *veinlet*, in which all of the longitudinal veins are normally shortened (Fig. 3). One vein could be selected to be shorter, while another vein was selected to be longer. This vein-specific action was also reflected in the contributions of different chromosomes. Chromosome II might carry polygenes affecting one vein while chromosome III would carry polygenes affecting another. This is consistent with the concept of individual polygenes acting on components of some developmental process. Polygenes acting specifically on the *veinlet* locus would have been expected to modify all veins in the same way.

But the demonstration of 'region-specific' effects makes an even more important contribution to the problem of polygene function and number. It underlines the fact that one is really concerned with a phenotype which is a composite of many separate, though integrated, processes. The longitudinal veins in the *Drosophila* wing are, to a degree, genetically and developmentally independent. This is seen in the fact that some mutations affect only one vein[24]. Thus, in considering polygene number, one should really be concerned with the complexity of the phenotype—the number of component processes which polygenes could influence.

This point can be illustrated further by the sternopleural bristle system. Spickett[25] isolated three polygenic loci which accounted for the majority of the differences between a selected high bristle number line and a control stock. He

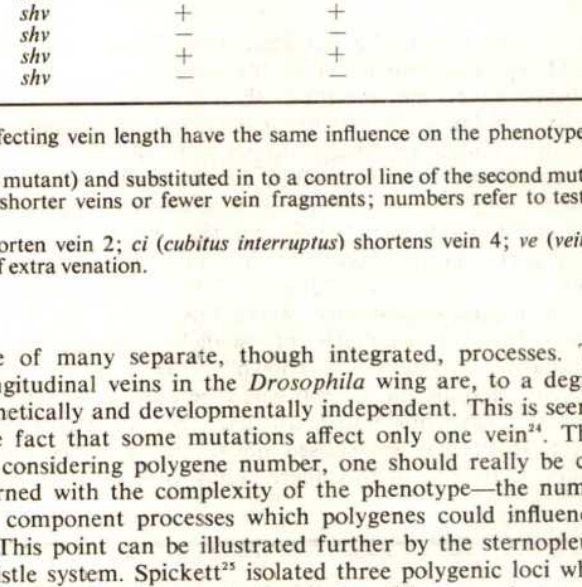

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Fig. 3 Representative phenotypes from two *veinlet* selection lines, in which vein lengths were modified in different directions. *a*, The L4 was selected shorter, and the others were selected to be long. *b*, The L3 was selected to be long, and the others were shortened. Vein-specific polygenic effects were responsible for the responses to selection[23].

then looked at the developmental effects of each of these loci. One caused a general increase in cell number, indirectly increasing the number of bristle-forming cells. One increased the distribution of small bristles on the ventral part of the sternopleurum. The third locus altered the timing of cell division of one large dorsal bristle, causing two bristles to be formed in its place. Each of these loci affected a different developmental process, but directly or indirectly contributed to the variation in sternopleural bristle number. A similar situation was described by Jacobson[26] who found that selection for increased facial vibrissae number in mice was caused by the development of extra hair follicles, whereas back selection affected not the number of follicles but the suppression of their formation of hairs. Variation in two quite different processes was involved. In a complex character, such as body weight, one could imagine the number of contributing processes to be even larger.

The more 'complex' a character, the more polygenes one should expect to influence it. Unfortunately, such a criterion is based more on intuition than on judgement. But the expectation is, I believe, logical, and it is consistent with our limited knowledge of polygene function.

Even though polygenic effects are numerous, the actual number of loci is probably of the same order as the number of 'major mutant' loci. If it is true that polygenic loci and major gene loci are essentially the same, this implies that many polygenes are isoalleles of known major mutants. Certain technical problems[27] make this difficult to test in most cases, but examples of isoalleles contributing to quantitative variation are known[18,28]. In a sense, then, polygenes may be the normal wild-type alleles.

We still have much to learn about the variety of gene effects which may be classed under the term 'polygenic variation'. In spite of this, there seems to be no compelling reason to believe that the total number of loci affecting quantitative characters is incompatible with estimates of gene number based on major mutant complementation studies.

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articles

Metamorphic belt and volcanic arc migration in New Zealand

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The Phanerozoic development of New Zealand can be viewed in terms of four eastward migrating cycles of closely similar character, all of which are related to westward dipping Benioff zones. Paired metamorphic belts are produced during the final stages of each cycle.

It is now well established that the Cretaceous Rangitata Orogeny in New Zealand produced two parallel belts of contrasting metamorphism: a western belt characterised by granites, and an eastern, low temperature, higher-pressure belt characterised by wide zones of low grade metamorphism[1]. According to plate tectonic theory this pairing would have developed over a westward dipping Benioff zone. Similarly, the Permian, Cretaceous, Tertiary and Recent volcanic arc structures in New Zealand have been related to westward dipping Benioff zones[2,3].

I show here that it is possible to view the entire Phanerozoic development of New Zealand in terms of westward dipping Benioff zones, a view attractive for its simplicity. Four closely similar cycles can be recognised, each ending in an orogeny producing a paired metamorphic belt. Each new cycle is developed to the east of the preceding one.

Sedimentary belt migration

Three belts of more or less fossiliferous sediments can be recognised. The eastern margin of each belt in turn becomes the western margin of the younger belt so that they become progressively younger to the east (Fig. 1). The oldest (western) belt (equal to the central belt and eastern part of Cooper's western sedimentary belt[4]) contains Cambrian to Upper Ordovician sediments; the central belt (the eastern sedimentary belt of Cooper[4]) contains uppermost Ordovician to Devonian sediments; and the eastern belt (Hokonui facies) contains Permian to Jurassic sediments The Hokonui facies was derived from a landmass to the west[5], and was probably laid down on oceanic crust now represented by an ophiolite complex[6].

The source area and nature of the basement for the two older belts have not been established; there is no evidence of a continental basement. In each case, older rocks lie to the west and form a possible source, whereas there is no known source to the east.

Two other sedimentary belts of quite different character can be recognised. First, covering large areas to the west of the oldest fossiliferous belt is a monotonous sequence of sparsely fossiliferous Cambrian to Ordovician[7,8] turbidites (Fig. 1) which were laid down on a Precambrian continental crust[8]. Their character and subsequent metamorphic history differs from that of the similarly aged sediments in the oldest fossiliferous belt. Second, to the east of the youngest fossiliferous belt is a widespread and thick succession of sparsely fossiliferous sediments, mainly of Carboniferous to Jurassic age (Torlesse Supergroup) (Fig. 1). Unlike the similarly aged Hokonui facies, these sediments were derived from the east[9], and may be completely allochthonous, possibly having been conveyed from the east by seafloor spreading and welded to the Hokonui Belt during the Cretaceous Orogeny (ref. 10 and J. D. Bradshaw personal communication).

Non-orogenic volcanic arc migration

Several volcanic arcs have been recognised in New Zealand. A distinction can be made between non-orogenic arcs and those associated with orogeny and granite formation.

The non-orogenic volcanic arcs migrate progressively eastwards with time (Fig. 2). The oldest andesitic arc of Cambrian age borders the east side of the oldest fossiliferous sedimentary belt[4]. Volcanics interlayered with sediments of Ordovician–Silurian age occur on the east side of the central sedimentary belt[11], but their character is obscure since they are invaded by Cretaceous granite; the massive Rotoroa Complex (Fig 2) of basic igneous rocks lies in this belt, but it too is metamorphosed by Cretaceous granite, and ages as various as Cambrian to Cretaceous have been suggested. Late Carboniferous diorite and more basic intrusions also occur on the east side of the central belt[11,12], again in direct line with the Rotoroa Complex Further east, Permian volcanic arcs—one has been interpreted[6] as an ophiolite complex (see Fig. 2)—were developed in association with sediments of the Hokonui facies. Finally, between the Rangitata and the present Kaikoura orogeny, a late Cretaceous volcanic arc developed in the exotic Torlesse Belt[3].

The oldest four arcs are associated with and parallel to the fossiliferous sedimentary belts of the same age. It may be speculated that if the allochthonous Torlesse Supergroup had not piled up against the Hokonui facies, the Cretaceous arc would similarly have been associated with a belt of late Cretaceous fossiliferous sediments laid down to the east of the autochthonous New Zealand land mass.

The chemical variation in the Cretaceous and Permian arcs has been ascribed to a westward dipping Benioff zone[3]

Metamorphic belt migration

Four main periods of folding or orogeny can be recognised in New Zealand. a Miocene–present day Kaikoura Orogeny, a late Jurassic–mid Cretaceous Rangitata Orogeny; a Devonian–Carboniferous Tuhua Orogeny; and a late Ordovician period of folding and granite intrusion (as yet unnamed).

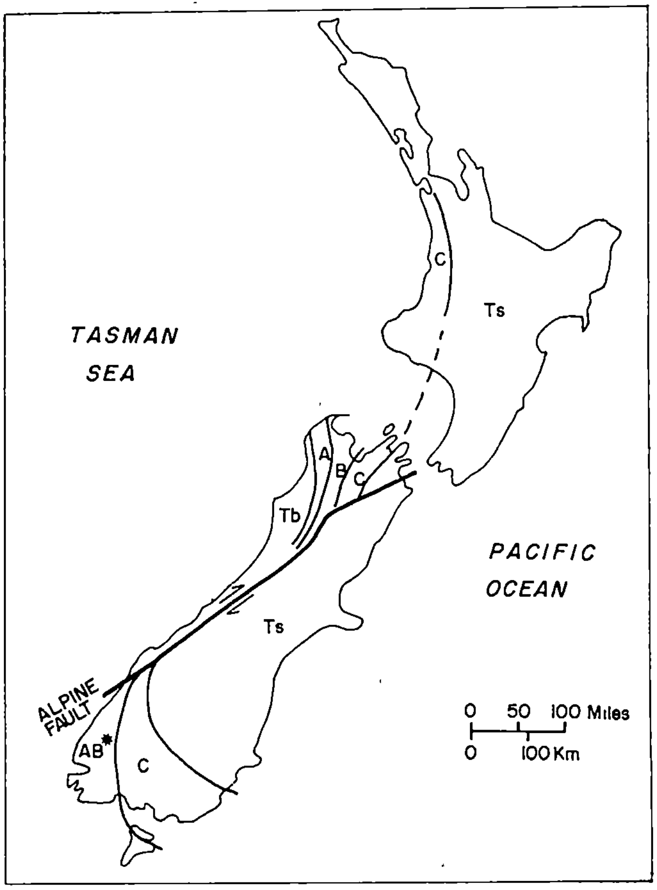

Fig. 1 *a*, *b* and *c*, Belts of more or less fossiliferous sedimentary rocks: *a*, Cambrian–Upper Ordovician; *b*, Upper Ordovician–Devonian; *c*, Permian–Jurassic. Tb, Area of Cambrian–Ordovician turbidites; Ts, rocks of the allochthonous Torlesse Supergroup. *ab**, belt which, because of intense Cretaceous metamorphism, cannot be differentiated in Fiordland.

As with the modern Mizuho Orogeny of Japan, the metamorphic effects of the Kaikoura Orogeny are difficult to judge. The opening of the Tasman Sea in late Cretaceous times[13], the development of the important transcurrent Alpine Fault, and the general fragmentation of Gondwanaland since the Mesozoic, have created what is probably an atypical asymmetry in the effects of the Kaikoura Orogeny. A Benioff zone dips westwards only under the North Island, and the submarine trench off the east coast similarly dies out southwards. It is reasonable to suppose that a paired metamorphic belt is developing under the North Island, as in present day Japan[14]. The high temperature belt is represented by successive volcanic arcs from the Miocene to the present (developed mainly where sediments of the Torlesse Supergroup crop out) which produced granites and voluminous ignimbrites, which are presumably the superficial expression of granite. According to plate tectonic theory, the trench zone represents a developing area of high pressure metamorphism. The median boundary of the paired belts must lie parallel to the eastern coast of the North Island, to the east of the ignimbrite belt (Fig. 3).

The median boundary for the Rangitata Orogeny is well documented[1], and lies between the old New Zealand landmass and the post-Tuhuan, Hokonui facies sediments (Fig. 3). The paired belt with granites to the west of the median boundary indicates subduction from the east.

Median boundaries for the Tuhua and pre-Tuhua Orogenies have not previously been proposed, but there is

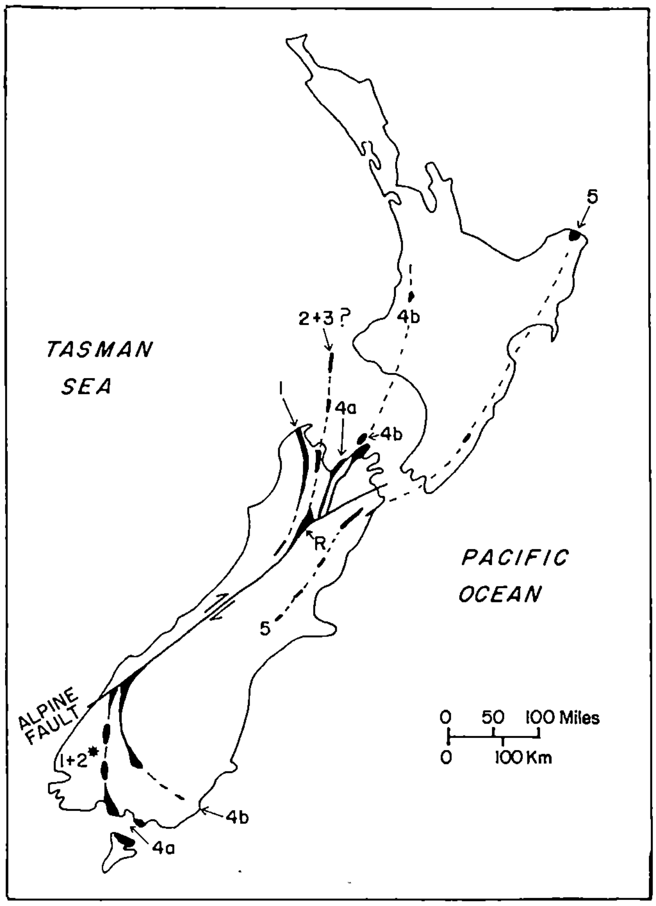

Fig. 2 Non-orogenic volcanic arcs: 1, Cambrian; 2, Upper Ordovician–Silurian; 3, Carboniferous; 4, Permo–Trias (4b may be Permian ophiolite[6]); 5, Upper Cretaceous. Fiordland geology; (1+2*), not yet elucidated. Extension of 2+3 offshore based on drillhole data[24]. R, Rotoroa Complex.

good evidence that older boundaries do exist to the west of, and parallel to, the younger boundaries.

Granites of Tuhuan age abound in the west and northwest of the South Island[15], and produce andalusite–sillimanite hornfelses at their contacts. The most easterly Tuhuan granites intrude the Cambrian–Ordovician sediments of the western belt. Tuhuan deformation of the Upper Ordovician–Devonian sediments in the central sedimentary belt (Fig. 1) was accompanied by the formation of wide zones of low to moderate grade schists (up to staurolite grade) which commonly contain almandine. Although the formation of the schists has been regarded as resulting from[16,17] the intrusion of Cretaceous granites, that view is erroneous. Andalusite and sillimanite have formed at the immediate contacts of the granites, and the thermal effect has been superimposed on already existing almandine schists; if the low pressure Cretaceous metamorphism had produced the schists, andalusite should appear in the assemblages before almandine, which it does not. The superimposition of the thermal effect is supported by textural studies near Springs Junction (S. W. Quek, personal communication). The voluminous uppermost Ordovician marbles contain abundant biaxial calcite (N. Newman, personal communication) which has been interpreted[18] as a possible indication of inversion from high pressure aragonite. The general aspect in this belt is, therefore, of moderate to high pressure metamorphism. In common with modern orogenic belts, it seems that a Tuhuan median boundary separating granite activity from a belt of higher pressure metamorphism can be located close to the junction of the old landmass and the pre-orogenic sedimentary belt (Fig. 3).

The pre-Tuhua Orogeny is even less well documented than the Tuhuan, but recent radiometric work provides good evidence for the position of a median belt. The slaty cleavage formation in the sparsely fossiliferous Cambrian–Ordovician sediments (Fig. 1) is now dated at 440 Myr (ref. 19) which place it in the Upper Ordovician[20], and which precisely correlates with the migration of sedimentary deposition from the western to the central belts. The slaty cleavage was induced by the gravity sliding of wet (or dehydrating?) sediments in association with granite intrusion and a high geothermal gradient[21,22]. Granites older than Tuhuan (about 450 Myr old) occur along the western coast of South Island (C. J. D. Adams, personal communication); their eastern limit is unknown, but is viewed here as coinciding with the eastern limit of the common structural pattern in the cleaved sediments. The fossiliferous sediments of the western belt (Fig. 1) underwent a complex deformation before the formation of the central belt. This deformation has generally been ascribed to the Tuhuan Orogeny[11], but in view of the absence of the early phase of folding in the sediments of the central belt, I regard the deformation as Ordovician (the only evidence that the deformation is Tuhuan is the presence of supposed but undated uppermost Ordovician marble slivers along the base of some thrusts[11]). Large areas of low grade schist with abundant stilpnomelane in some assemblages[23] indicate a relatively high pressure metamorphism. The pre-Tuhuan median boundary separating granites on the west and higher pressure metamorphics on the east can therefore be drawn to the west of the Tuhuan median boundary (Fig. 3).

The metamorphic belts for both Palaeozoic orogenies

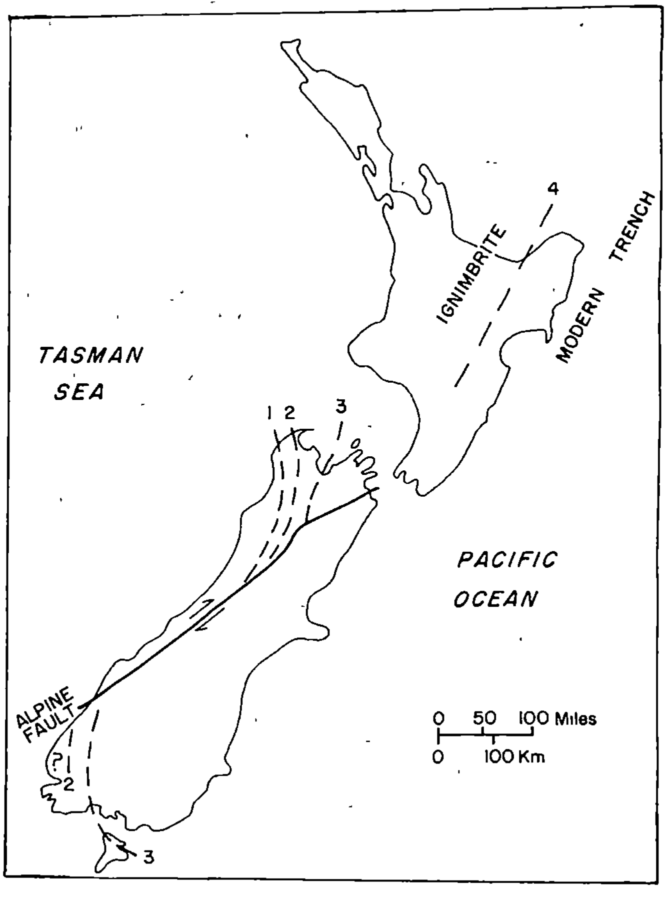

Fig. 3 Median boundaries of paired metamorphic belts, separating low pressure granite activity on the west from higher pressure metamorphism (without granites) on the east: 1, pre-Tuhuan Orogeny; 2, Tuhua Orogeny; 3, Rangitata Orogeny; 4, Kaikoura Orogeny.

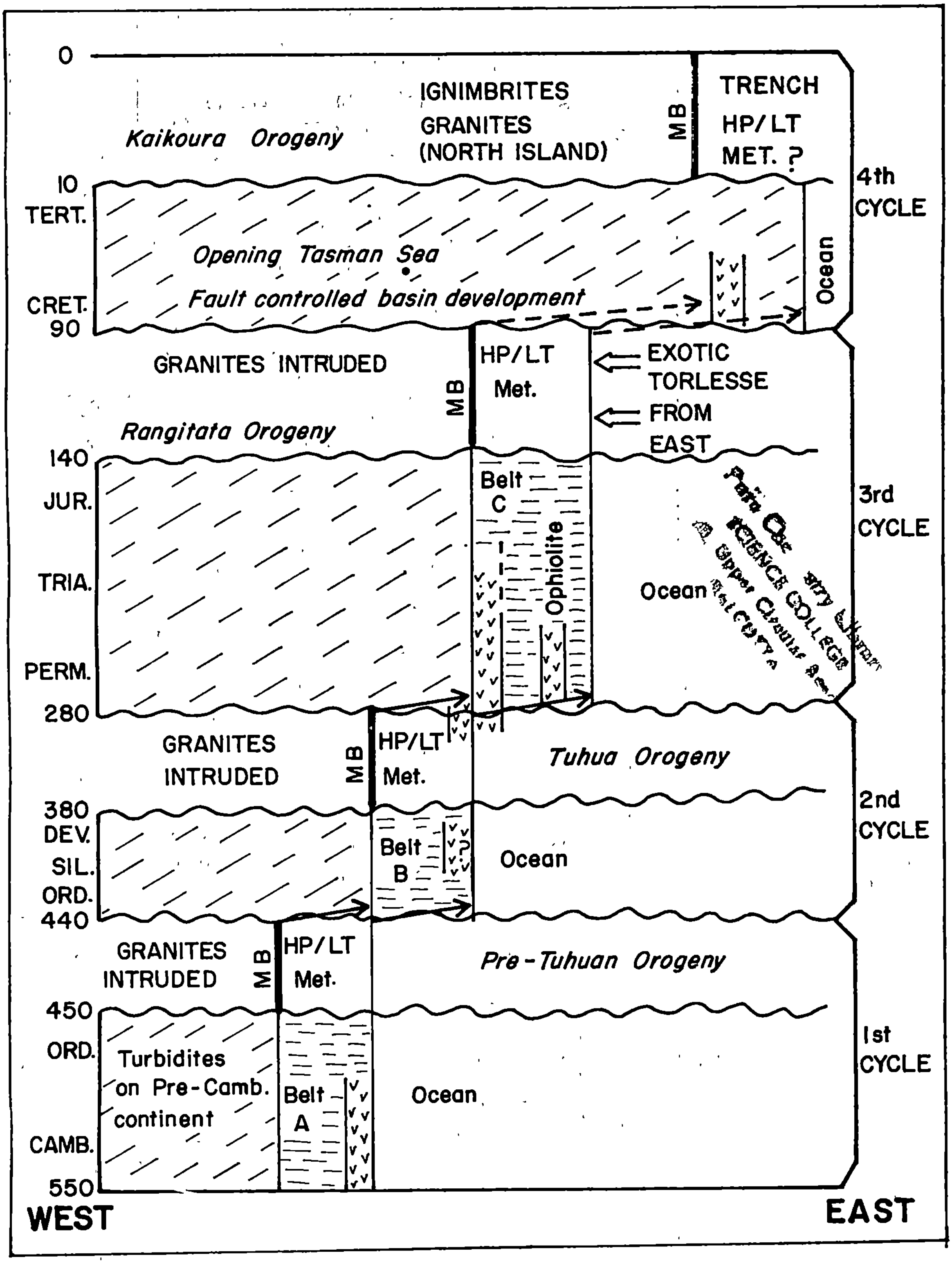

Fig. 4 Diagram summarising in time and space the cycles of sedimentation, non-orogenic volcanic arc formation and paired metamorphic belt formation. MB, Median boundary of the paired metamorphic belts. Belts A, B and C as in Fig. 1; V, non-orogenic volcanic arcs.

run parallel to the Rangitatan and modern trends, and the pairing with granites to the west and higher pressure metamorphics to the east, consistently indicates a westward dipping Benioff zone.

Eastward migrating cycles ending in orogeny

It is possible (see Fig. 4) to recognise a common cycle of events, repeated successively at more easterly locations. It seems that the entire Phanerozoic history of New Zealand can be related to a series of Benioff zones dipping westwards under the New Zealand landmass. Each cycle involves three stages. First, deposition of a fossiliferous sedimentary belt directly to the east of a continental landmass (except for the latest cycle, which has been interfered with by the introduction of the allochthonous Torlesse Supergroup, and the fragmentation of Gondwanaland); second, a pre-orogenic volcanic arc or arcs associated with the sedimentary belt; and third, an orogeny producing

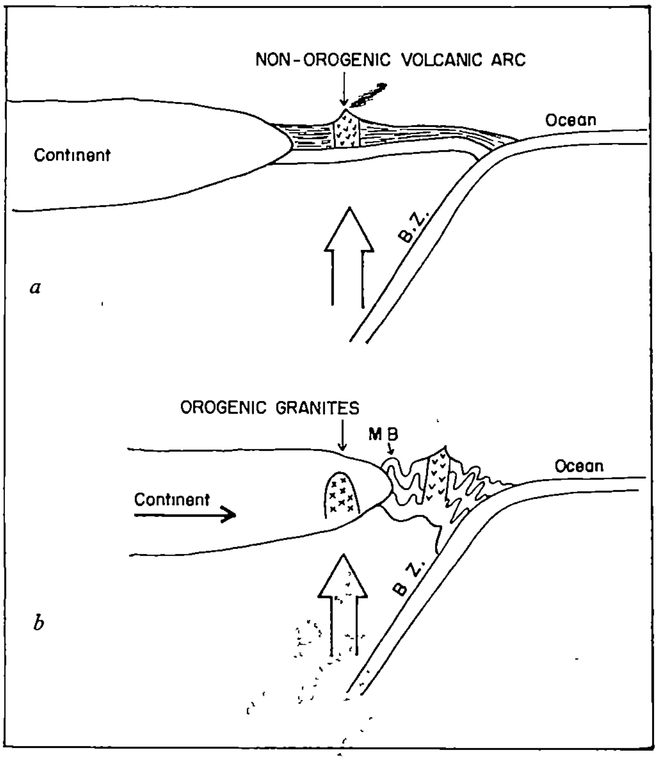

Fig. 5 Possible explanation for the relative positions of the non-orogenic volcanic arcs and orogenic granite belts. See text for discussion. BZ, Benioff zone; MB, median boundary of the paired metamorphic belts.

a paired metamorphic belt, the median boundary lying between the old landmass and the sedimentary belt.

The fossiliferous sedimentary belts probably all have a basement of oceanic crust which may be represented in at least one belt by an ophiolite complex[6]. Each new cycle represents the initiation of a new Benioff zone parallel to, but east of, the previous zone.

An important implication is that an ocean, like the Pacific, has existed to the east of New Zealand since at least the Cambrian. Japan shows a similar parallel development of paired metamorphic belts from the Palaeozoic to the present[14].

Relationship of volcanic arcs to metamorphic belts

The standard picture of a Pacific orogenic belt, as deduced, for example, from Japan[14], is that of a volcanic arc coinciding with the granitic metamorphic belt of high temperature/low pressure. In New Zealand, it seems that between orogenies the arc lay in the pre-orogenic sedimentary belt destined to suffer a high pressure metamorphism (Fig. 4); during the ensuing orogeny, the arc becomes transposed relatively to the west to lie under the old landmass. An explanation may be that an accelerated spreading rate during orogeny causes the landmass to compress and override the sedimentary rocks to the east, so that the high temperature effects are apparently transposed westwards (Fig. 5); in other words, it may be the continent that moves rather than the zone of high heat flow.

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Summer phytoplankton blooms and red tides along tidal fronts in the approaches to the English Channel

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Tidal mixing on the continental shelf in the South-western Approaches to the English Channel produces a situation in the summer where within a few miles the warmer surface water becomes completely mixed with the underlying colder water. These frontal boundaries may be followed for 100 nautical miles. By combining physical and biological oceanographic disciplines it has become clear that these boundaries are the sites of phytoplankton blooms which, in favourable conditions, may develop into red tides.

Our understanding of primary production in the sea seems to be hampered by an inadequate knowledge of the turbulent nature of the physical environment in which the phytoplankton exist. Progress in quantifying the distribution and growth rates of marine phytoplankton has taken two main directions; that of modelling[1–3], and the more statistical approach relating the variance and coherence of temperature and chlorophyll *a* to horizontal space scales[4–6]. Useful as the modelling approach has been in general, its main weakness is the uncertainty involved in specifying the mixing coefficients, which for the shelf region in the Western Approaches to the English

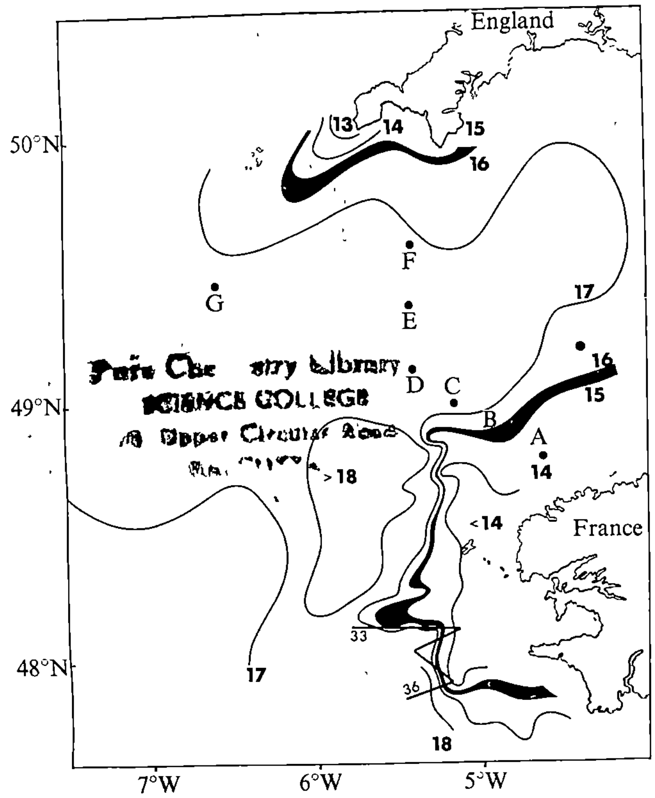

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Fig. 1 Sea surface temperature contours obtained between July 26 and July 30, 1975. The 15–16 °C contours define the shelf front or the outcropping of the seasonal thermocline at the sea surface.

Channel may vary from 1 to 10^7 cm^2 s^{-1} (refs 7 and 8). The statistical approach is also a potentially valuable technique, but mechanisms of mixing which may be important in determining the distribution of phytoplankton are easily obscured. Here we show that the distribution and production rates of phytoplankton in the approaches to the English Channel are fundamentally linked with the turbulent nature of the environment. At this early stage a simple description of the possible operative mechanisms involved may be more illuminating and basic to our understanding than any attempt to define diffusion parameters or the statistical properties of the environment.

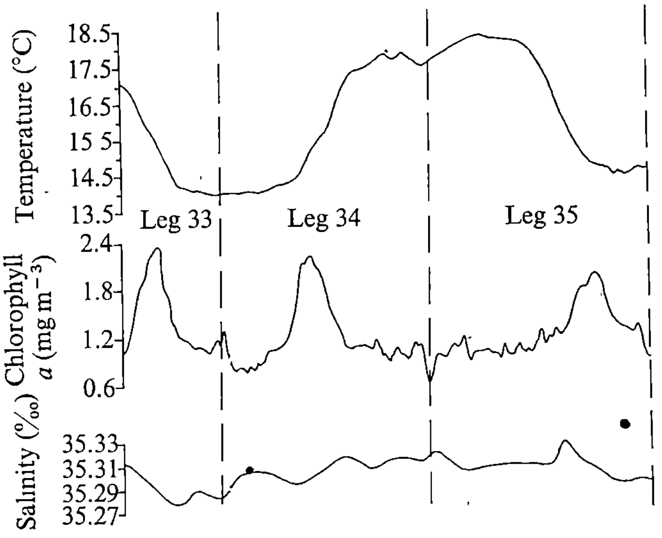
Surface temperature and chlorophyll *a*

It is surprising that for an area like the English Channel, where hydrographic data have been collected for nearly a century, and for which mean monthly temperature distributions were available in atlas form 40 yr ago[9], large surface temperature discontinuities that may be followed for up to 100 nautical miles (Fig. 1) have not been described. These structures mark the transition between well stratified shelf water and well mixed coastal water, or the outcropping of the thermocline at the sea surface. Stratification nearer the shore is also often evident, caused predominantly by warm, less saline water running off the land. Around the coast of France the transition can be defined by the maximum surface temperature gradient and in July 1975 was represented by the 15–16 °C surface temperature contours. In June 1974, it was similarly positioned and readily identifiable between the 13 and 14 °C contours[10]. With hindsight this feature can be recognised in earlier work[11], but it is, of course, more recent techniques, giving continuous records of hydrographic data, that have helped to define these regions clearly, and allow unambiguous interpretations that are often not possible with the more traditional methods based on sampling at discrete stations. Examples of continuous traces of temperature, salinity[12] and chlorophyll *a*[13] can be seen in Fig. 2. These traces were obtained south of Ushant (legs 33, 34 and 35, see Fig. 1) where the frontal characteristics were particularly well developed[14–16]. Here about 50%(~1.5 °C) of the temperature change across the region occurred within 3,000 m. Patches of floating weed were often evident in this zone[17] and also conspicuous was the frequent presence of fog over the cooler water. The lack of any large salinity changes (<0.03‰) or consistent correlations of salinity and temperature suggest that advection of warm surface water may not in general be responsible for the summer surface temperature distribution in the Western Approaches although historically the role of advection, rather than local surface heating and vertical mixing (over horizontal scales of the order of the tidal excursion), has been assumed dominant[18]. The chlorophyll *a* trace does, however, show a clear association with the temperature gradient. It is this association between temperature anomalies and phytoplankton distribution within the general framework of the presented hydrography, and the special circumstances that may bring about summer plankton blooms, and even red tides, which we are particularly concerned with here.

Vertical structures of temperature and chlorophyll *a* through a region of phytoplankton bloom

The vertical profiles of temperature and chlorophyll *a* were more conveniently investigated where the transition zone between well mixed and stratified regimes was broad, so that movement of the front itself during the measurements presented fewer difficulties. Indeed, on one station North-west of Ushant the water column appeared with a 3 °C temperature inversion, without a corresponding stabilising salinity change, reflecting frontal movement. The temperature and chlorophyll *a* data shown in Figs 3 and 4 can be placed geographically by reference to Fig. 1. Here the maximum surface temperature gradient between stratified and well mixed regimes occurs in the neighbourhood of station B. Station G has been included as representative of the extensive area of stratified water that is established on the shelf in the Western Approaches during the summer months. High values of chlorophyll *a* occur in surface waters in the frontal region. These extend well into the stratified side, and are also observed in the thermocline. The visual correlation with the temperature section is conspicuous. It would be misleading, however, to suggest that the phytoplankton distribution is controlled by temperature. The common factor producing these distribution patterns is water turbulence.

Fig. 2 Continuous surface temperature, salinity and chlorophyll *a* traces obtained south of Ushant (see Fig. 1, legs 33, 34 and 35). Note that the chlorophyll *a* trace responds with the temperature gradient (or perhaps more correctly near where the second differential of temperature with respect to time (distance) takes a positive sign).



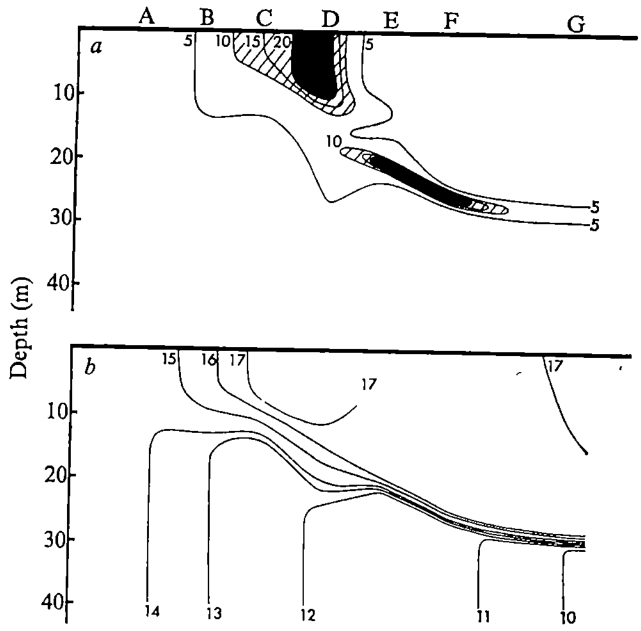


Fig. 3 *a*, Chlorophyll *a* section through the frontal region. The units of chlorophyll *a* concentration are mg m^{-3}. *b*, The corresponding temperature section. The units are in °C. Station positions A, B, C, D, E, F, G are as shown in Fig. 1.

In the stratified region three different mixing regimes can be identified, namely, the top mixed layer, the thermocline and the bottom mixed layer. The turbulence in the top mixed layer is derived from wind stress and night-time convection. The turbulent kinetic energy production has its maximum input near the surface. In the bottom mixed layer the turbulent energy is derived mainly from the tides, but near the shore, swell motion may also make a signficant contribution. In this region the turbulence declines away from the sea floor. The thermocline forms between the two mixed layers when the wind above the tide below can no longer stir the excess surface heating downwards. Here the ratio of the buoyancy flux to the turbulent energy production rate (that is, the local flux Richardson number) will be a maximum and the water column tends to stabilise, further inhibiting mixing. Towards the coast of France the tidal stream amplitude increases (Table 1) and the increased production rate of turbulent kinetic energy from below reduces the depth at which the thermocline can develop. The temperature section of Fig. 3 therefore represents the gradual change from the complete overlap which excludes the development of the thermocline to the situation where the wind and bottom mixed regimes are well separated vertically.

In general, differences in salinity will also contribute to the stability of the thermocline. The relative importance of salinity and temperature to the buoyancy flux can be determined from the ratio $\beta\Delta S/\alpha\Delta T$ where α is the coefficient of thermal expansion, β the coefficient of saline contraction and ΔT and ΔS are the corresponding temperature and salinity differences across the thermocline. This ratio was generally less than 5% (see Table 1) and so low that the temperature structure (Fig. 4) can also be considered as representative of the density structure.

In the stratified region, plant production in the surface layer is restricted by low levels of inorganic nutrients (Table 1, stations E, F, G). Values of chlorophyll *a* are also low below the thermocline, in spite of higher nutrient levels, because the mean light level is much reduced (generally less than 1% of the surface values). Why then are the values in the intermediate region in the thermocline as much as forty times larger than those above or below? Usual interpretations are based on phytoplankton aggregations due to sinking and settling on density discontinuities[19], or active downward swimming of flagellates towards the source of nutrients[20]. By considering a time scale for mixing, it can be illustrated that the interaction of the turbulence level with the levels of light and nutrients may largely determine the distribution characteristics of the chlorophyll *a* profiles.

Dispersal of phytoplankton in well mixed and stratified regions

Estimates for the lifetime of small scale structures in the sea have led to some interesting speculations concerning their generation and dissipation[21,22]. In a similar manner it is useful to have a diffusive time scale for the chlorophyll *a* structures in the water column. A characteristic time for a layer to mix above and below with its surroundings depends on the layer thickness, H, and the diffusion coefficient, K. A time for mixing, t, may be defined by

$$t \sim H^2/4K$$

by analogy with the classical problem in heat flow, where t then represents the time taken for a uniformly heated layer to lose half its excess heat into a medium with the same conductivity[23].

In the bottom mixed layer the dominant stirring action of the tides produces a homogeneous temperature region in which the temperature gradient approaches the adiabatic value[24]. If close to the bottom, the diffusion coefficient may be written as[25]

$$K = 0.4u_*H$$

where the friction velocity u_* is defined from the bottom stress, τ_B, by $\tau_B = \rho u_*^2$, ρ is the density of seawater and H is the

Fig. 4 Vertical profiles of chlorophyll *a* and temperature obtained in: *a*, the well mixed (A); *b*, frontal (C); and *c*, stratified regions (E). The values are the original uncalibrated data which may be fixed absolutely by reference to Fig. 3.

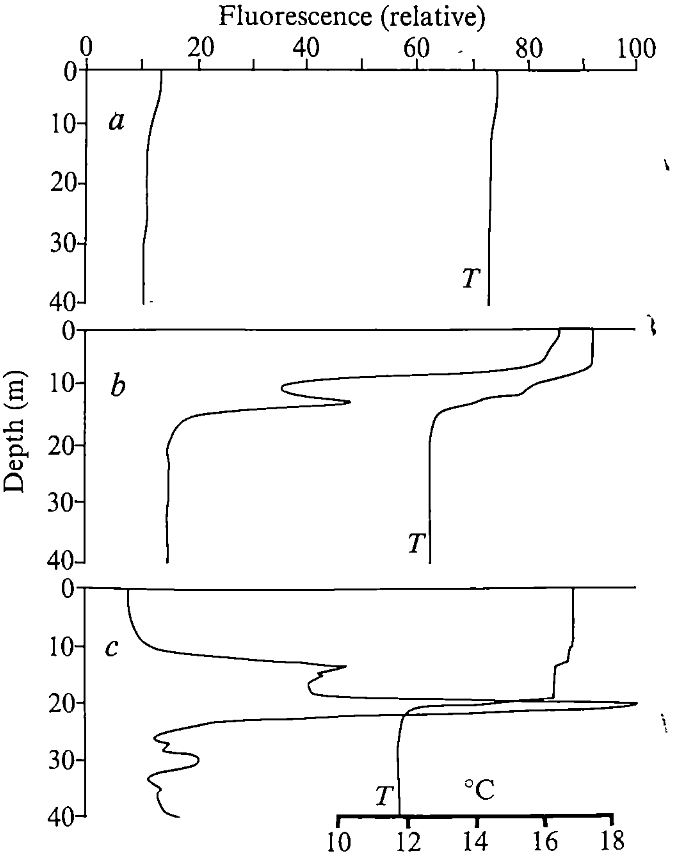


Table 1

Stations	A	B	C	D	E	F	G
Water depth (m)	92	98	107	108	106	94	110
Tidal stream amplitude at springs (m s^{-1})	1.3	1.1	0.9	0.8	0.7	0.7	0.6
Surface minus bottom temperature (°C)	0.2	2.7	5.1	5.7	5.3	5.4	7.5
Surface minus bottom salinity (‰)	0.01	0.024	0.065	0.060	0.043	0.067	0.074
Buoyancy flux ratio ($\beta\Delta S/\alpha\Delta T$) (%)		4	5	4	3	5	3
PO_4—P Surface layer 2 m	0.41	0.37	0.43	0.49	0.28	0.16	0.18
(µg atom l^{-1}) Bottom layer 40 m		0.39	0.39	0.42	0.50	0.61	0.73
NO_3—N Surface layer 2 m		2.56	1.79		0.1	0.69	0.25
(µg atom l^{-1}) Bottom layer 40 m		0.58	1.39		3.2	0.85	1.39
SiO_3—Si Surface layer 2 m	0.95	0.89	1.26		0.56	0.26	0.59
(µg atom l^{-1}) Bottom layer 40 m		0.85	0.74		0.63	0.93	1.23

height above bottom, then a mixing time scale for the bottom region of height H is

$$t \sim H/u_*$$

Here the validity of this relationship away from the bottom has been extended for illustrative purposes for an order of magnitude estimate. The bottom stress was determined from current meters placed 1, 2 and 3 m off the bottom at position 49°27′N, 4°42′W over a period of a month where the tidal streams were similar in amplitude to those in the neighbourhood of stations D and E. The semi-major axis of the semi-diurnal lunar, M_2, friction velocity was ~ 2 cm s^{-1} (ref. 26). This gives a time scale for mixing in this region below the thermocline of the order of 1 h during maximum tidal streaming. This is small in comparison with the division rate of phytoplankton cells[27] and does not permit a chlorophyll-rich zone to become established below the thermocline where the light intensity is still favourable. Furthermore the efficiency of mixing in this region explains why the small scale structures in the chlorophyll *a* profiles in the region below the thermocline (Fig. 4*c*) are transitory and not generally reproducible from profile to profile. The mixing efficiency in the bottom layer makes hypotheses concerning the migration of plant cells into nutrient-rich water less attractive since these cells risk being rapidly dispersed in the same manner as heat. Similar calculations can be made for a time scale in the wind-mixed layer, but as conditions at the sea surface are unpredictable the derived mixing values are less meaningful.

A time scale for the thermocline can be formed from

$$t \sim \frac{H^2\delta T/\delta z}{4F_h}$$

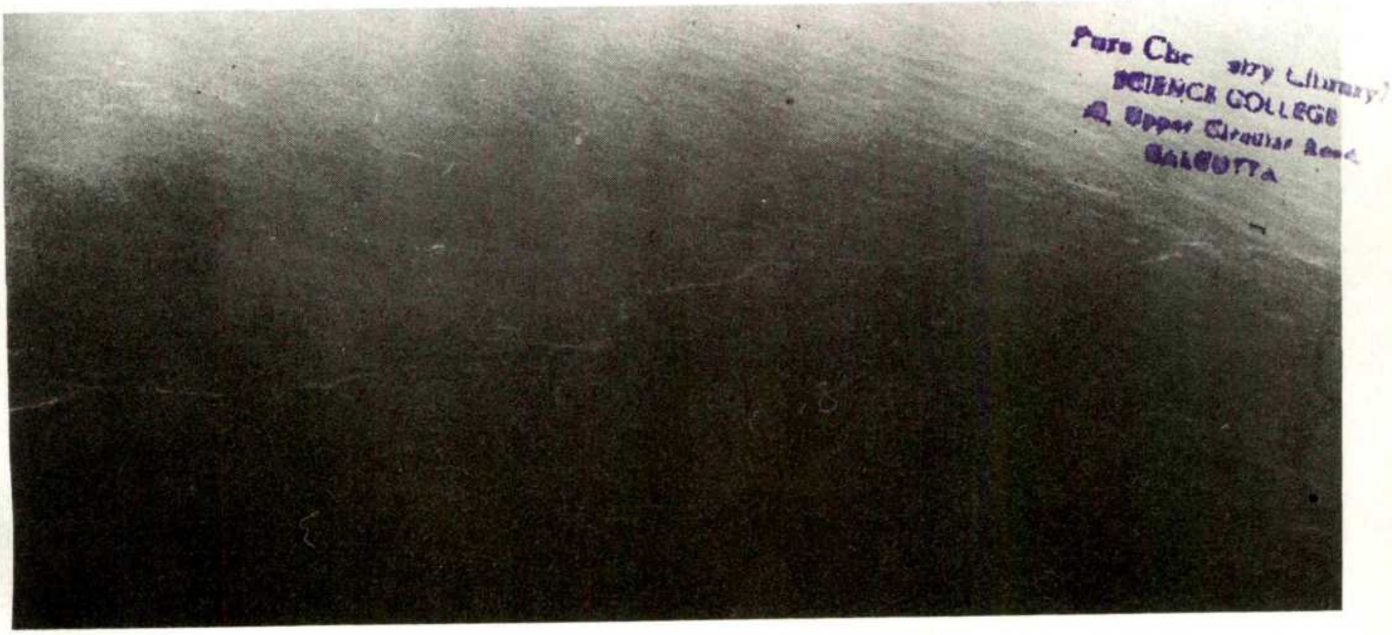
where F_h is the heat flux penetrating a thickness H of the thermocline of temperature gradient $\delta T/\delta z$

or
$$t \sim \frac{H(\theta_2 - \theta_1)}{4F_h}$$

where $\theta_2 - \theta_1$ is the temperature difference across the region H. Substituting typical values, $H = 5$ m, $\theta_2 - \theta_1 = 4$ °C and $F_h = 2$ kcalorie cm^{-2} $month^{-1}$ (based on the warming rate of the bottom mixed layers), gives a time scale t, of about a week. This is large compared with the cell division rate of most phytoplankton. Thus, higher cell concentrations in the thermocline may simply reflect the reduced tendency for their dispersal by turbulence. The large time scale in the thermocline is consistent with the persistence of the chlorophyll *a* maximum in the thermocline from station to station. Cells in this region would also be first in line for nutrients brought up from below in the same manner that heat is continually mixed downwards.

In the well mixed region (Fig. 4*a*) there are both adequate light and nutrients (Table 1) for cell growth at the surface. The thoroughness of the mixing (or the short time scale), however, does not give the plant cells sufficient time in the surface layers to produce any marked degree of surface phytoplankton

Fig. 5 Aerial photograph from 6,000 feet (August 7, 1975; 1400 GMT) showing red tide effects in the neighbourhood of station C. Surface wind light and variable; sea state—low swell.



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concentrations here. In a similar manner, vertical structures in both chlorophyll *a* and temperature profiles are conspicuously absent in this region.

Conditions favouring summer plankton blooms

The frontal region represents an area of recently stabilised mixed water in which the combination of high nutrients and a shallow upper mixed layer create conditions suitable for the rapid growth of phytoplankton[28]. These conditions are particularly dependent on weather and tide. At spring tides, for example, the tidal stream amplitude is approximately double that at neap tides, with a corresponding cubed production rate of turbulent kinetic energy in the bottom mixed layer, causing the well mixed region to increase in size at the expense of the more stratified region and nutrients to be brought to the surface. As the tidal streams slacken towards neaps, the surface water stabilises and conditions are set for a plankton bloom. In a similar manner, a period of turbulent weather will cause the frontal boundary to move into the stratified region, particularly if this is also the wind direction. Indeed a continual series of blooms may be created by the interaction of weather and tide as the balance between stabilisation and increasing turbulence is altered along this shallow thermocline region. It is worth remarking that it is the shallowness of the thermocline in this region that largely determines the size of the area likely to be effectively mixed and subsequently to produce favourable growth conditions. Our observations were made during calm weather following a period of spring tides and strong winds, which perhaps accounts for the large size of the region in bloom condition. The high surface values for dissolved inorganic nutrients at stations C and D, which exceeded those at 40 m (Table 1), cannot yet be explained.

Red tide characteristics within bloom region

Although so far we have not found it necessary to consider the swimming activities and buoyancy effects of the phytoplankton cells it is evident that they must be important on smaller scales within the bloom region. Dominant in the frontal region was the dinoflagellate *Gyrodinium aureolum* Hulburt, a species that has been reported as occurring in high concentrations in other areas[29–31]. Counts of a representative sample (34 mg chlorophyll *a* per m^3) gave the following values (cells per l $\times 10^6$): *G. aureolum*, 1.7; *Prorocentrum micans* Ehr., 0.01; flagellates, 1.4; and all other photosynthetic cells, 0.005. Areas were found where the concentration of chlorophyll *a* was as high as 100 mg m^{-3}. A week later these reddish-brown streaks were observed from a height of 6,000 feet (Fig. 5), which were aligned in parallel but broken and irregular rows typical of a red-tide phenomenon[32]. A conservative estimate[33–35] would suggest these patches contain more nutrients than were available in the water, and it seems unlikely that they could be produced without any interaction between cell movement and water turbulence. Concentrating mechanisms might be based on a daytime upward migration[43] coupled with weakly organised Langmuir circulations[36], or on the interaction between phytoplankton movement and internal waves[37]. Red tide effects have also been reported from inshore regions, though these are often due to positively buoyant organisms such as *Noctiluca*[38,39].

Primary production in characteristic regions

Interesting as these distributions of phytoplankton standing crop are, they are not a measure of primary production in the various regions. Rates of carbon dioxide fixation were determined by inoculating appropriate water samples with NaH^{14}CO$_3$ and incubating them at sea surface temperature under fluorescent lights in the ship's laboratory or *in situ* under natural light at various depths. The data are summarised in Table 2. Mean values for chlorophyll *a* are representative for the vertically mixed regime in the neighbourhood of station A, for the frontal region between stations B and D, and for the top and bottom mixed layers in the stratified region. The thermocline is considered to be between 25 m and 30 m, and a mean value for chlorophyll *a* across the most concentrated 5 m of the chlorophyll peak is given. The assimilation index (AI)[27] is the potential for carbon fixation under light saturation (equivalent to 10% midday summer sunlight)[40]. High indices were found for plant populations in the thermocline and in the vertically mixed regime. Lower indices for other regions may reflect a lack of dissolved nutrients or a poor physiological condition of the cells. In the frontal region the assimilation index seems to be inversely related to cell concentration, and for a higher chlorophyll *a* concentration of 78 mg m^{-3} a value of 1.34 mg carbon per h per mg chlorophyll *a* was observed.

Rates of primary production *P* were calculated from the equation

$$P = \mathrm{AI}\ \text{chlorophyll}\ a \int_0^z I_0 e^{-kz} \mathrm{d}z$$

(where I_0 is subsurface light intensity and z is depth of the water column) and expressed relative to a value of unity for the vertically mixed regime. In the stratified regime, for which the relative production rate is greater than one, the phytoplankton in the thermocline account for at least 50% of the total carbon fixation. Some caution is necessary in specifying this proportion since it will vary with light intensity. For example, under high surface light intensities photosynthesis will be maximal in the thermocline but saturated or light-inhibited in the top mixed layer. The production occurring below the thermocline seems relatively unimportant. In the frontal region, in spite of both the high extinction coefficient and the low assimilation index, the relative production is high. Thus, it seems that this weakly stratified region has an important bearing on primary production in the English Channel in the summer, and, furthermore, it is notable that the turbulent structure is also important in determining the rate of production of phytoplankton as well as the distribution pattern for the standing crop.

It is clear that further studies on primary production and the distribution of chlorophyll *a* in other associated frontal systems in the English Channel, for example, around the Lizard and Land's End (see Fig. 1), are necessary. Further work also needs to be directed towards answering questions such as which of

Table 2 Primary production data

	Mixed	Frontal	Stratified		
			Wind mixed layer	Thermocline	Bottom mixed layer
Chlorophyll *a* (mg m^{-3})	0.45	19.0	0.55	9.5	0.66
Extinction coefficient *k* (m^{-1})	0.12	0.34	0.10	$0.1 < k < 0.3$	0.10
Assimilation index (AI) (mg C fixed per h per mg chlorophyll *a*)	4.2	1.9	2.2	5.7	0.2
Relative production rate (*P*)	1.0	6.5	0.6	0.7–1.1 1.6	0.05

the three regions, well mixed, frontal or stratified, is generally the most productive; what are the relative contributions of the top mixed layer, thermocline and bottom mixed layer to the total production of the water column in the stratified regime; and how representative is a single station, like E1 (ref. 41), of production in the English Channel? The interrelationship between the distribution of phytoplankton and herbivorous zooplankton is poorly understood, particularly for the stratified regime. It seems probable that the high concentration of plant cells in the thermocline is an important food source for both migratory[42] and non-migratory grazing organisms.

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Models for metal ion function in carbonic anhydrase

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A model catalyst is described which has properties in common with carbonic anhydrase. The model demonstrates the availability of a mechanism, previously only hypothetical, for the action of the enzyme. It also shows, however, that this mechanism alone is not adequate to produce the high activity of the enzyme.

DETECTED in 1928, discovered in 1932, recognised to contain zinc in 1939, obtained pure in 1959, crystallised in 1962 and the subject of some 1,800 papers and articles, carbonic anhydrase continues to elude a full mechanistic description, even though this enzyme is small, stable and easily isolated, and its X-ray structure is known to a resolution[1,2] of 2 Å.

Carbonic anhydrase catalyses a range of reactions, including the hydration of carbon dioxide and aldehydes, and the hydrolysis of carboxylic, sulphonic and carbonic esters. The CO_2 hydration is apparently the reaction of biological importance, both in respiration and in intracellular CO_2–HCO_3^- equilibration. In most of these reactions the rate in the absence of enzyme is believed to be limited by the nucleophilic attack of an oxygen atom or ion at an electrophilic centre, usually carbon, illustrated in Fig. 1*a*. The enzyme accelerates the CO_2 hydration, the acetaldehyde hydration and the hydrolysis of *p*-nitrophenyl acetate by factors of 10^9, 10^5 and 10^5 respectively over the reaction in water.

The zinc ion which occurs naturally in carbonic anhydrase may be replaced artificially by other metal ions, but only cobalt(II) and zinc confer appreciable activity on the enzyme; with no metal or with other metals the enzyme is practically inactive in hydration.

The enzyme possesses an ionising group with a pK_a of about 7; only the alkaline form of the enzyme is catalytically active.

One elegant mechanistic suggestion[3] is that a metal-bound water molecule could ionise to provide the necessary nucleophile in the form of metal-bound hydroxide (Fig. 1*b*). Other possibilities include the ionisation of a nearby histidine group to provide a general-base catalyst at the active site[4]; the zinc could then act as a point of substrate binding, perhaps also polarising and thereby activating the substrate by virtue of its Lewis acid strength[5]. Whatever the mechanism of nucleophilic attack, other stages of the reaction may also be catalysed, such as proton transfer within the enzyme–substrate complex[6] or between the enzyme and its aqueous environment[7].

The purpose of this article is to re-examine the plausibility of the first mechanism referred to above, the zinc-bound hydroxide nucleophile (ZHN) hypothesis. Reviews and background literature are given in refs 2 and 8.

A priori arguments against the ZHN mechanism have been twofold: first, that a zinc-bound water cannot have such a low pK_a as 7 (refs 4 and 9); second, that a zinc-bound hydroxide ion would be so polarised by the zinc as to lose most of its negative charge to the zinc and thus be too weak a nucleophile to attack, for example CO_2 (refs 6 and 10 and Fig. 1*b*). Transition-metal hydroxy complexes can indeed attack CO_2 (refs 11 and 12), but so far few quantitative data are available.

To investigate the universality of these two presuppositions, model systems were chosen to reflect closely the desired properties of the enzyme. The much-abused term "model" is here understood as a simple chemical system with a property or properties relating it to the biological system under consideration. Ideally a range of model compounds, structurally controlled to reproduce in varying degrees the property in question, provide a quantitative scale on which the biological system

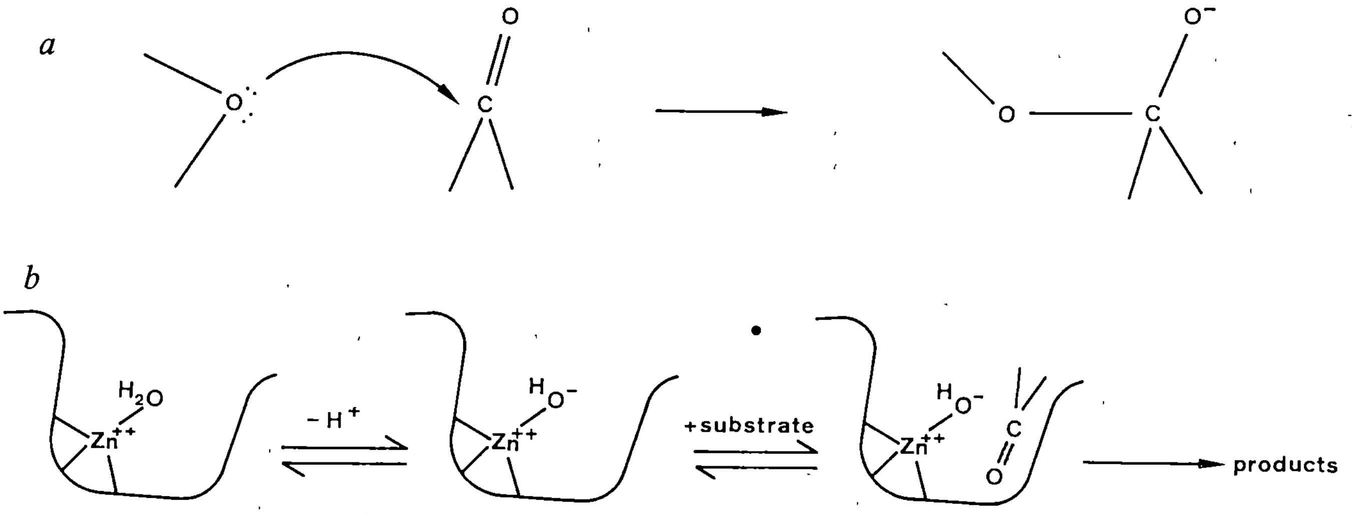

Fig. 1 Nucleophilic attack. *a*, Negative charge on oxygen enables it to react through its outer electron pairs with a positive centre such as the carbonyl carbon shown here; *b*, a postulated mechanism for nucleophilic attack in carbonic anhydrase.

might fall; if it continues to deviate from this scale then it has still not been fully understood. A model system may alternatively be designed to reproduce one of the biological properties of the system, for instance, to test the hypothesis that a sufficient condition for the action of an enzyme is a particular conjunction of reacting atoms or groups; if successful this does not confirm that the biological system acts in the same way as the model, but it does show that the action of the simple system is in principle available for nature to make use of in the complex one.

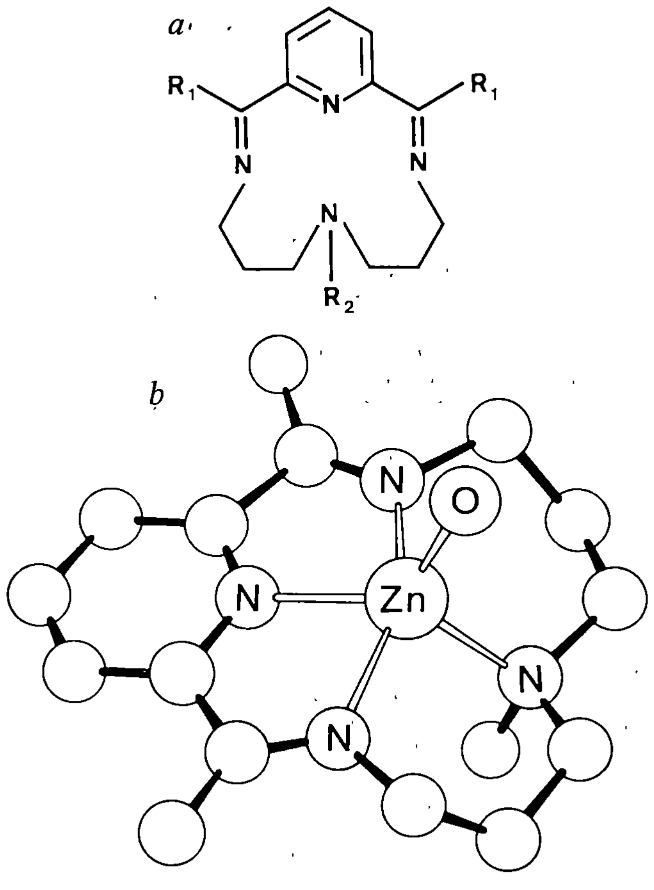

Fig. 2 Macrocyclic ligands. *a*, Free ligand. *b*, In the complex the four nitrogen atoms bind a metal ion—Zn^{2+}, Cu^{2+}, Ni^{2+} or Co^{2+}—which also binds water; the skeleton of the $Zn(N\text{-}MeCR)(H_2O)^{2+}$ complex is shown (H omitted, unlabelled atoms are C.) CR: $R_1 = CH_3$, $R_2 = H$; N-MeCR: $R_1 = R_2 = CH_3$; *des*diMeCR: $R_1 = R_2 = H$.

An ionised water molecule?

Discussion of factors influencing the ionisability of water bound to zinc is bedevilled by the possible formation of hydrolysed cluster species and by the uncertain coordination number of the metal ion when water molecules are counted[13,14], as well as by the complexity of the equilibria present[14,15]. To overcome these problems, macrocyclic ligands of the type CR (ref. 16 and refs therein) (Fig. 2) were used. Here the four nitrogen donor atoms are perforce bound to the metal, giving room for one or two water molecules. Electronic spectroscopy[16] and X-ray crystallography[17] support coordination numbers in aqueous solution of five for $CoCR^{2+}$ and $ZnCR^{2+}$, and six for $NiCR^{2+}$ and $CuCR^{2+}$.

Titration of the complexes in aqueous solution with sodium hydroxide reveals the pattern of behaviour shown in Table 1; two points emerge. First, the metals which can activate carbonic anhydrase can also promote the ionisation of water in their CR complexes. If this is due to the reduced coordination number of zinc or cobalt then the same ionisation should be possible in carbonic anhydrase. Second, nickel and copper do not promote this ionisation, although they are normally stronger Lewis acids, forming stronger complexes, than zinc or cobalt (II) (for example ref 18). A tempting conclusion is that whatever chemical influence determines the ionisation pattern of the CR complexes must also operate in carbonic anhydrase. This is, however, a *non sequitur;* a more logical approach is required.

The stronger acidity of five-coordinate $ZnCR^{2+}$ and $CoCR^{2+}$ than that of their corresponding hexaquo ions is probably determined by their reduced coordination number, as can be argued on simple electrostatic grounds[13,19], reducing the overall coordination number increases the total positive charge on zinc and the resulting polarisation of the bound H_2O. A further reduction in pK_a when the coordination number is reduced to four, as found in carbonic anhydrase[1], would therefore not be surprising. So far, carbonic anhydrase is the only zinc complex known for certain to have a coordination sphere composed of three nitrogens plus one oxygen in solution, although there are other candidates[20] for this. Similar titrimetric investigations using acyclic polyamine ligands point to the same correlation between coordination number and pK_a, (refs 21 and 22) with the additional insight that a less polar environment for the complex encourages ionisation still further, perhaps to the extent of 1–2 units, as plainly shown by methylation of the NH_2 groups of the polyamine[23]. This factor could well operate at the relatively non-polar active site of the enzyme. The provision of a firm binding site for zinc, of low coordination number and low polarity, and accessible to substrate, could indeed be the reason for the surprisingly large size of the protein in comparison with the substrate.

The probable higher coordination of the copper and nickel CR complexes similarly explains their lesser polarising effect on bound water molecules. Such a coordination number would reflect the known lesser propensity of zinc and cobalt, in comparison with copper and nickel, towards the formation of geometrically regular complexes, and indeed this very property, which imparts a greater coordination flexibility to Zn^{2+} and Co^{2+}, has been speculatively put forward as determinative of the activity of carbonic anhydrase with zinc and cobalt[24]. The active site of carbonic anhydrase appears to be fairly accommodating to the geometry preferred by the metal (ref. 25 and ref. 2, 642–643). Following this argument, one could say: if the activity in carbonic anhydrase is determined by an ionising metal-bound water molecule, then the non-activity of Cu^{2+} and Ni^{2+} in carbonic anhydrase could be a consequence of the non-ionisability or non-existence of this water, the latter a consequence of the coordination number taken up by Cu^{2+} or Ni^{2+} as the resultant of the different geometries preferred by the metal and the apoenzyme respectively. This must however be regarded as an hypothesis to be investigated and not as an explanation, particularly in view of the existence of an (as yet unidentified) ionising group in the inactive Mn^{2+}-, (ref. 46) Cu^{2+}- (E. Grell and A. McConnell, personal communication) and $(VO)^{2+}$- (ref. 27) substituted enzymes.

Table 1 Ionisation of water bound to metals in macrocyclic complexes, in aqueous solution at low ionic strength (0.002–0.04 mol l^{-1})

$$\mathrm{M(ligand)OH_2}^{2+} \overset{K_a}{\rightleftharpoons} \mathrm{M(ligand)OH^+} + \mathrm{H^+}$$

Ligand	M	Temperature (° C)	pK_a*	Approximate heat of ionisation (kcalorie mol^{-1})
CR	Zn	25	8.69†	7.1±1
CR	Zn	0	9.17	
CR	Cu	25	>11	
CR	Ni	25	>11	
CR	Co	25	8‡	
N-MeCR	Zn	25	8.12	8.3±1
N-MeCR	Zn	0	8.68	
N-MeCR	Cu	25	>11	
*des*diMeCR	Zn	25	8.13	6.0±1
*des*diMeCR	Zn	0	8.53	
$(H_2O)_5$	Zn,Co	—	~10	
Apocarbonic anhydrase	Zn,Co	—	~7	

* $pK_a = -\log_{10}K_a$. Error estimated from several determinations <0.05 units.
† This supercedes the value given in ref. 19.
‡ Estimated value; apparent cluster formation prevented a more accurate determination.

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The heat of ionisation of bovine carbonic anhydrase, 7 kcalorie mol^{-1}, has been taken as an indication that the ionising group is histidine, whose imidazole group has a similar heat of ionisation[28]. The data in Table 1 shows that this value is equally compatible with the ionisation of zinc-bound water. The point of emphasis here, however, is the potential stability of a zinc-bound hydroxide ion, irrespective of whether it is formed by dissociation of a water molecule or, for example, by the addition of hydroxide accompanying a conformational change of the protein.

A zinc-bound hydroxide nucleophile?

The purpose of the kinetic studies here was to test the second presupposition above—that metal-bound hydroxide is too poor a nucleophile to have a catalytic role in carbonic anhydrase. In addition, it was felt desirable to see what sort of modifications short of complexation by apocarbonic anhydrase would induce catalytic activity of any kind in zinc ions. The aim was thus to find a catalyst containing zinc ions along with organic groups and/or the hydroxide ion.

Carbonic anhydrase's range of substrates is wide, so it was necessary at the start to restrict the range of reactions examined. One reaction was studied intensively—the hydration of acetaldehyde. This was chosen as it is very efficiently catalysed by carbonic anhydrase and for reasons of experimental convenience.

The basic mechanism of acetaldehyde hydration has been well reviewed[29].

$$CH_3CHO + H_2O \underset{k_-}{\overset{k_+}{\rightleftharpoons}} CH_3CH(OH)_2$$

In contrast to carbonic anhydrase, aqueous zinc ions are only poor catalysts for the reaction, but there is a very simple, general catalytic effect in which zinc ions and other species in solution work together to catalyse the hydration reaction[30]. These other species include in particular hydroxide ions, and this effect was investigated further in the hope that it might also throw some light on the ZHN hypothesis. Unfortunately, the cooperation between zinc and hydroxide ions is less amenable to systematic mechanistic study, as practical difficulties are caused by polynucleation and precipitation. To study this reaction further it was therefore necessary to use a different system containing zinc and hydroxide, and the natural choice was the $ZnCR^{2+}$ complex, which was already available and whose properties were well investigated.

The efficiency of a catalyst in acetaldehyde hydration is defined as dk_+/d [catalyst]. Experimental details of the procedure used to measure the rates are given elsewhere (ref. 30 and refs therein). At low pH the efficiency of $ZnCR^{2+}$ is low, but at high pH it becomes very high (Fig. 3). This behaviour superficially resembles that of carbonic anhydrase[31].

Mechanism of $ZnCR^{2+}$ catalysis

The efficiencies of various catalysts are shown in Table 2. Empirically, $ZnCR^{2+}$ is a poor catalyst (Fig. 3, low pH) and $ZnCR.OH^+$, the form of the complex predominating at high pH, a good one (Fig. 3, high pH). Thus the $ZnCR^{2+}$ and OH^- cooperate to produce an enhanced catalysis, just as hoped for. A detailed analysis of the kinetics and activation parameters (to be published) shows that the hydration proceeds through collision between $ZnCR.OH^+$ and CH_3CHO. Two possible mechanisms for this are shown in Fig. 4. Borate is a more powerful base than $ZnCR.OH^+$, yet its catalytic effect is much smaller (Table 2). Therefore $ZnCR.OH^+$ cannot simply be acting as a general base (Fig. 4*a*), as then borate would be the better catalyst; it must be attacking the carbonyl group directly (Fig. 4*b*). Space-filling models suggest that simultaneous binding to zinc of hydroxide and aldehyde is forbidden by the geometry of the CR ligand; this point is being further investigated.

Table 2 Catalytic efficiency in acetaldehyde hydration

Catalyst	Efficiency	Reference
CH_3COO^-	0.023	30
CH_3COOH	0.073	
$Zn^{2+}{}_{aq}$	0.0084	
H_2O	0.00014	
$ZnCR^{2+}$	0.036	This work
$CuCR^{2+}$ (*p*H 5.0, 7 5)	0	
$CuCR^{2+}$ (*p*H 9.5)	2 ± 1 (?)	
$ZnCR.OH^+$	196	
$Zn(N\text{-}MeCR)OH^+$	112	
Zn(*des*diMeCR)OH^+	130	
OH^-	58,000	32
Carbonic anhydrase	14*	31
Borate	$\lesssim 7$	This work

Units are mol^{-1} l s^{-1}.

*The turnover number (700 s^{-1}) of the enzyme is divided by 50 mol l^{-1}, to obtain an order-of-magnitude correction for the presumed high concentration of catalyst at the active site.

To obtain a comparison, the catalytic properties $CuCR^{2+}$, $NiCR^{2+}$ and $CoCR^{2+}$ were examined in the same way. Unfortunately the nickel and cobalt complexes reacted directly with the acetaldehyde (shown by ultraviolet spectroscopy) and so no conclusion could be reached in these cases. $CuCR^{2+}$ was unaffected by the acetaldehyde and showed no catalytic properties at *p*H values of 5.0, 7.5, or 9.5. This is consistent with the absence of a $CuCR.OH^+$ species in this *p*H range, demonstrated by titration (Table 1).

That the catalysis in the presence of CR involves direct attack of zinc-bound hydroxide backs up the hypothesis that the same attack occurs in the above-mentioned cooperative catalysis by zinc and hydroxide ions.

Carbon dioxide hydration

The availability of zinc-bound hydroxide as a good nucleophile provides an obvious next step for investigation of its catalytic ability in other reactions, particularly those catalysed by carbonic anhydrase. Most interesting would be the hydration of CO_2, for which carbonic anhydrase's catalytic power is the greatest. This was examined qualitatively by mixing saturated CO_2 solutions with buffered solutions of $ZnCR^{2+}$ at 0 °C. The initial rate of *p*H change was measured and converted by comparison with an HCl titration to the rate of liberation of protons; this gave the reaction rate, for the stoichiometry is

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+$$

at the *p*H used (9.2). The empirical catalytic constant of the $ZnCR.OH^+$ complex is given by

$$\frac{1}{[CO_2]} \cdot \frac{d[CO_2]}{dt} \cdot \frac{1}{[ZnCR.OH^+]} \qquad (3)$$

and was determined as about 25 mol^{-1} l s^{-1}. Since previous work has demonstrated the absence of general-base catalysis in the hydration of CO_2 (refs 33 and 34) the most obvious mechanism available for attack is analogous to that of Fig. 4*b*. $ZnCR.OH^+$ is quite a good catalyst (Table 4), although still not comparable with carbonic anhydrase.

A similar result has been reported[12] using the complex {Cu.glycylglycinate.OH}, in which the active species was likewise considered to be a metal-bound hydroxide ion. $ZnCR.OH^+$ is a less ambiguous catalyst to interpret, as no potentially active ligand groups other than OH^- are present, and the metal has no further free coordination sites.

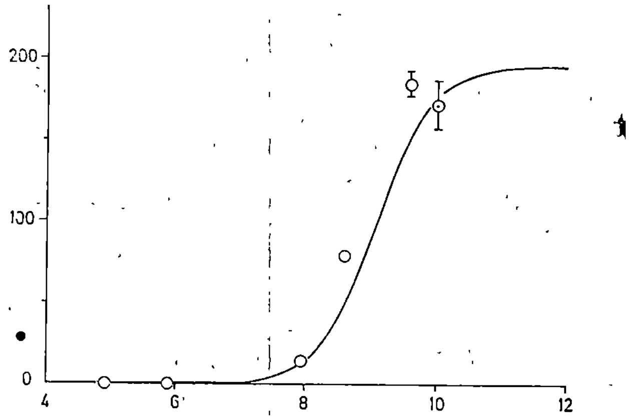

Fig. 3 The catalytic efficiency of $ZnCR^{2+}$ in the hydration of acetaldehyde, as a function of *p*H. The solid line is the curve calculated assuming that the active species is $ZnCR.OH^+$ with an efficiency of 196 mol^{-1} l s^{-1}. The pK_a found is 9 07 (compare the value from titration, 9.17, Table 1). Increasing errors at high *p*H made measurement above *p*H 10 impossible.

Relationship between model and enzyme

The applicability of the enzyme model to the enzyme can now be considered in relation to the ZHN hypothesis.

The titrimetric studies on $ZnCR^{2+}$ and related complexes, and similar studies on a range of polyamines (refs 22, 23 and unpublished), make it clear that the ionisation of zinc-bound water is to be expected, on condition that the coordination number of zinc be reduced from six to five or four—a condition fulfilled by carbonic anhydrase. This does not prove that the ZHN in carbonic anhydrase exists, but it means, should a zinc-bound water in carbonic anhydrase be shown not to ionise, that this in itself would be a remarkable property of the enzyme, and in need of special explanation.

Table 3 Catalytic efficiency in carbon dioxide hydration

Catalyst	Efficiency	Reference
$H_2As_2O_3^-$	0.9	33*
$HAsO_4^-$	0.002	
BrO^-	2	
SeO_3^-	1.2	
SO_3^-	0.2	
NO_2^-, NO_3^-, CO_3^{2-}, SO_4^{2-}	0	
Complexes MX_n, where M=Mn, Fe, Co, Ni, Cu, Zn^{2+} and X=EDTA, nitrilotriacetic acid, dipyridyl, cysteine, mercaptoethylamine and cyanide	0	34*
Cu(glycylglycinate)OH	20	12
$ZnCR.OH^+$	25	This work
Carbonic anhydrase	10,000†	35

Units are mol^{-1} l s^{-1}.

*In these references different units are used. The data have been converted so as to accord with the definition given in this article.

†See footnote to Table 2

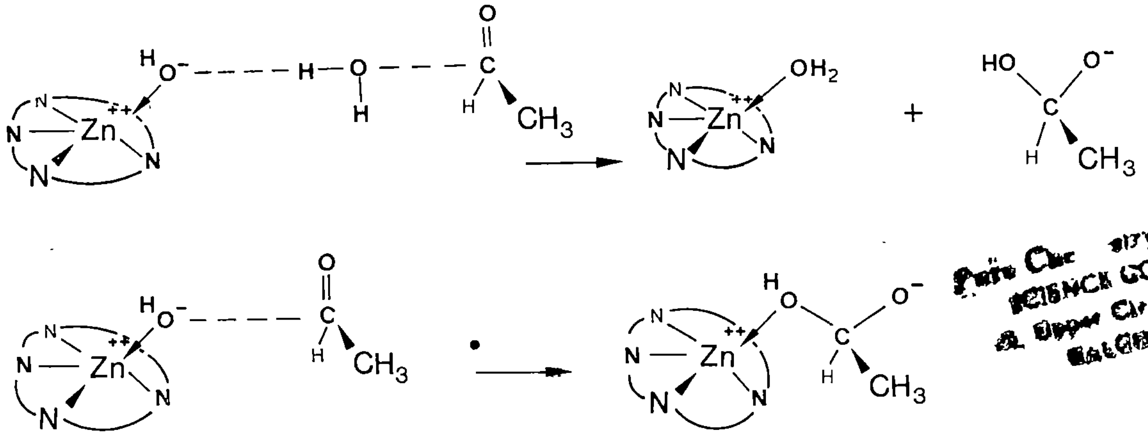

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Fig. 4 *a*, General-base and *b*, direct (nucleophilic) attack by ZnCR.OH$^+$. Only the rate-determining step is shown; proton transfer and ligand replacement at zinc are assumed to be fast.

The kinetic work has shown that a zinc-bound hydroxide ion can act as a very powerful nucleophile, even though its proton affinity is about 10^7 times that of a free hydroxide ion. Its nucleophilicity is great enough to account for the enzyme's catalysis of acetaldehyde hydration, though not of carbon dioxide hydration (Tables 2 and 3). The existence of this nucleophile in the enzyme and in the model does not mean that the mechanisms in the enzyme and in the model are the same; indeed, the rates for CO_2 are so different that, even if there is a ZHN in carbonic anhydrase, the enzyme must still have an extra means of assisting the hydration which the acetaldehyde, because of its different structure, cannot utilise. There is also evidence[36,37] that an additional, tightly-bound bicarbonate ion could have such a role by mediating proton transfer in the zinc–substrate complex.

The evidence for the ZHN postulate on the basis of enzyme studies is far from overwhelming; model studies cannot add to it directly, although they appear to increase the previous plausibility and the comprehensibility of such a mechanism. What happens when one tries to extend the hypothesis to the metal-substituted carbonic anhydrases, with the thought that their activity or inactivity could be related to the presence or absence of an ionising water molecule at the metal? At first sight this seems reasonable. The flexible ions Zn^{2+} and Co^{2+} accept the distorted geometry and low coordination number offered by the enzyme, while copper and nickel do not, thus failing to polarise the bound water sufficiently to induce ionisation. Manganese is coordinatively flexible, but polarises bound water only weakly (it forms in general comparatively weak complexes[18], and in carbonic anhydrase seems to be less capable than zinc of polarising sulphonamide inhibitors[38]).

Before such a theory could be accepted, however, several obstacles would need to be removed. (1) Ionising groups are associated with metal in Mn^{2+}-, Cu^{2+}- and $(VO)^{2+}$-substituted carbonic anhydrases (see above). These would have to be shown not to be metal-bound water. (2) In the acid form of cobalt-carbonic anhydrase the metal ion is apparently very poorly accessible to exchanging water molecules[39]. Also, the spectroscopic change associated with the activity-linked ionisation in the cobalt enzyme is very marked[9], whereas the spectroscopic effects of water and hydroxide ligands are almost identical[40], and the spectrum of CoCR.OH$_2^{2+}$ is correspondingly similar to that of CoCR.OH$^+$ (unpublished). Thus, even if the ZHN hypothesis is correct, certain features of the metal's coordination geometry require clarification. (3) A kinetic effect remains unexplained. The forms of zinc-carbonic anhydrase with the more acidic proposed zinc-bound water molecules—having presumably the most strongly polarising zinc ions—should by virtue of the diminished negative charge on the hydroxide's oxygen atom possess the weakest nucleophiles, with the smallest rate of attack on substrates. The reverse order is seen, however[41,24]. This is also the case with the simple compounds ZnOH$^+_{aq}$ and ZnCR.OH$^+$ (Table 2) for the substrate acetaldehyde. Nucleophilicity and basicity are not always closely related[42], but a satisfactory explanation of this would still be welcome.

Conclusions from models

Models show that the presence of the postulated zinc-bound hydroxide ion in carbonic anhydrase at neutral *p*H values would be in accord with reasonable expectation. This hydroxide ion would have sufficient nucleophilic power to account for the enzyme's activity in acetaldehyde hydration, although not in carbon dioxide hydration. The ZHN postulate for the enzyme led to the observation of surprisingly high nucleophilicity in the zinc-bound hydroxide. Thus the uses and limitations of chemically modelling a biological system are illustrated.

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Conservation of cytoplasmic poly(A)-containing RNA in mouse and rat

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By comparing the melting temperature of DNA–DNA duplexes between related species, it is shown that total single-copy DNA evolves at a faster rate than DNA which is transcribed into poly(A)-containing RNA. Such a comparison suggests that at least 70% of rat single-copy DNA does not code for protein.

THE organisation and function of the DNA in the eukaryotic genome are the subject of much speculation and research[1,2] There exists a variety of models which concern themselves with the regulation of gene activity. At present, however, few data show what fraction of eukaryotic DNA is genetically active, that is, what fraction of the DNA codes for protein. An alternative way of posing the same question is: how many different proteins are synthesised throughout the life of the organism?

Various estimates of this number make distinctly different predictions Based largely on genetic analyses of *Drosophila* lethal mutations, the minimal estimate gives approximately 5,000 genes encoded by the *Drosophila* genome[3] Mammalian haploid genomes contain 3 pg of DNA[1]. Approximately 70% of the genome is single-copy DNA, known to be the site of transcription of most of the messenger RNA. Assuming a number-average molecular weight of 6×10^5 for eukaryotic mRNA[5], 5,000 different mRNAs (the minimal estimate for *Drosophila*) represent only 0.5% of the single-copy fraction of mammalian DNA. If the number of genes is proportional to the genome size, this estimate for mammals increases to 50,000 different mRNA species and 5.0% of the available single-copy DNA. From a consideration of the mutation rate observed for amino acid changes in protein, Kimura has concluded that the cost to a species due to deleterious mutations would be very high if all the DNA of eukaryotes were expressed genetically[4], in accordance with these low estimates of the protein coding fraction of the genome. Alternatively, there may be many more than 50,000 different mRNA species and, therefore, much more than 5% of the single-copy DNA of mammals may be protein-coding DNA. In the extreme,

Table 1 Extents of reaction and thermal stabilities of homologous and heterologous hybrids with rat ^{3}H-cDNA and rat ^{32}P-scDNA

		Homologous					Heterologous				
		Rat DNA	scDNA		cDNA		Mouse DNA	scDNA		cDNA	
Experiment	Reassociation temperature (°C)	% Rxn	% Rxn	ΔT_m (°C)	% Rxn.	ΔT_m (°C)	% Rxn	% Rxn.	ΔT_m (°C)	% Rxn.	ΔT_m (°C)
a	60	90	86	0.5	87	3.0	90	67	14.0	82	8.0
b	70	85	83	0.5	72.5	3.0	78	31	9.0	65	6.0
c	73	75	73	0.5	67	2.5	74	18.5	8.0	51	6.0
d	75	78	77.5	0.5	70	3.0	72	9.5	6.0	35.5	6.0
e	75	—	—	—	—	—	—	1.3	—	4.0	—
f	75	—	—	—	—	—	—	1.6	—	—	—

SI Nuclease

		Homologous				Heterologous			
		scDNA		cDNA		scDNA		cDNA	
	Reassociation temperature (°C)	% Rxn	T_m (°C)	% Rxn.	T_m (°C)	% Rxn	T_m (°C)	% Rxn	T_m (°C)
g	75	56	66.5	65	66 5	7	64.5	37	64 5

The data for experiments *a*, *b*, *d*, and *g* are taken from Figs 1, 2, 3, and 4, respectively. Experiment *c* is identical to experiments *a*, *b*, and *d* with reassociation temperature of 73 °C. In experiments *e* and *f*, radioactive tracer DNA was incubated in the absence of non-radioactive driver DNA as in Fig. 1.

10^6 different mRNA species represent 100% of the single-copy fraction.

A direct test of these two hypotheses is technically difficult It would be necessary to isolate and quantify all the different protein molecules (or mRNA molecules) synthesised in every tissue, at every stage of development throughout the lifetime of the organism. In experiments of this nature, it would be extremely easy to miss a relatively rare set of gene products. This class might well represent much, or even most, of the potential genetic complexity in the genome. Any number generated by direct counting of gene products in this manner is, therefore, perforce a lower limit.

We have now used an indirect approach to distinguish between these two extreme models By comparing the melting temperature of DNA–DNA duplexes between related species, Kohne[6] and Laird *et al.*[7] have shown that the rate of base substitutions is high in mammalian evolution. Similar studies of mRNA–cDNA duplexes (complementary DNA synthesised with reverse transcriptase) have shown that haemoglobin mRNA evolves at a rate considerably slower than single-copy DNA[8]. This observation, if true for most or all mRNA sequences, would suggest that the reduced base substitution between two related species (in this case *Mus musculus* and *Rattus norvegicus*), when compared with the base substitution in total single-copy DNA, could be used to estimate the percentage of single-copy DNA homologous to mRNA in these organisms. We have confirmed and extended this observation by examining the thermal stability of heterologous cDNA–DNA duplexes in which the cDNA was synthesised from total cytoplasmic poly(A)-containing RNA isolated from a tissue culture cell line, rat myoblasts. This cDNA, representing thousands of different messenger RNA sequences, was annealed in vast DNA excess to mouse DNA[9,10]. The comparison of the extent of reaction and thermal stability of these heterologous rat cDNA–mouse DNA duplex molecules with rat single-copy DNA–mouse DNA duplex molecules suggests that most of the single-copy DNA in these organisms does not serve as template for messenger RNA.

Single-copy DNA and cDNA homology

The relationship between thermal stability and degree of base mismatch of double-stranded DNA has been extensively investigated[11]. The mean temperature of elution of duplex DNA from hydroxylapatite (T_m), compared with an internal standard of perfectly matched duplex DNA, is an indication of the degree of base mismatch. A reduction in T_m (ΔT_m) is proportional to the number of mispaired bases present. When molecules from two related species are compared by hybridisation, this ΔT_m represents the proportion of nucleotides which have been substituted in the DNAs since their divergence from the most recent common ancestor. Rat ^{3}H-cDNA and rat ^{32}P-single-copy DNA (scDNA) were annealed with a vast excess of homologous (rat) or heterologous (mouse) DNA to a C_0t of 10^4 mol s l^{-1} in 0.24 M phosphate buffer (PB). At a temperature of incubation of 60 °C, 85–90% of the homologous reannealed DNA is retained by hydroxylapatite at 60 °C and elutes at a characteristic and reproducible temperature (Fig. 1*a* and *c*). The rat ^{32}P-scDNA, reannealed to cold rat DNA, elutes at the same temperature (±0.5 °C) as the reannealed non-radioactive rat DNA "driver" The ^{3}H-cDNA–DNA duplexes elute approximately 3 °C lower than the ^{32}P-scDNA–DNA duplexes The reason for this reduced melting profile of cDNA–DNA duplexes is not understood. It is possible that the cDNA has a low enough molecular weight to account for this result as its average size as determined by alkaline sucrose gradient sedimentation (370 nucleotides) is smaller than that of the scDNA and the non-radioactive

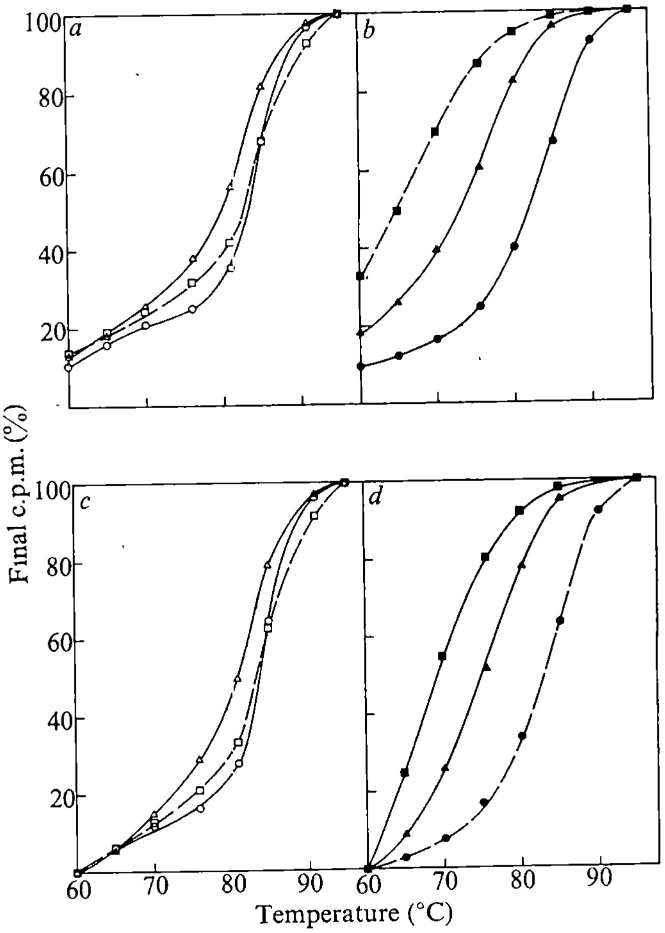


Fig. 1 Melting curves of hybrids of rat ^{3}H-cDNA and ^{32}P-scDNA to mouse and rat DNA incubated at 60 °C and assayed on hydroxylapatite. Rat ^{3}H-cDNA and rat ^{32}P-scDNA were hybridised to a vast excess (a ratio of at least 400) of mouse and rat DNA in 0.24 M phosphate buffer (PB) at 60 °C. The DNA, at 10 mg ml^{-1}, was denatured and incubated for 80 h, to a C_0t of ~ 10,000 in 0.24 M PB at 60 °C. 200 μg of DNA, containing approximately 5,000 c.p m. of both ^{3}H-cDNA and ^{32}P-scDNA was diluted to 0.12 M PB and applied at 60 °C to columns containing 500 mg hydroxylapatite and 500 mg cellulose. DNA was eluted with 3 aliquots of 10-ml 0.12 M PB at 60 °C. The temperature of the columns was raised in 5 °C intervals and the DNA eluted with 10 ml of 0.12 M PB at each temperature. A final 10-ml elution was performed with 0.4 M PB at 95 °C. Recovery from the column was 100±5%. The amount of driver mouse and rat DNA was determined by the absorbance at 260 nm of each sample ^{3}H-cDNA and ^{32}P-scDNA in each sample was measured by scintillation counting of samples after precipitation with TCA and BSA carrier. DNA, and cytoplasmic poly(A)-containing RNA, were prepared as described previously[16]. ^{3}H-cDNA was prepared from poly(A)-containing RNA with the reverse transcriptase of avian myeloblastosis virus. ^{32}P-scDNA was prepared by reassociation of total DNA to C_0t 200 in 0 24 M PB at 70 °C and removal of repeated sequences on hydroxylapatite. This 60% of the DNA, recovered in the single stranded fraction, displayed single-copy kinetics when rehybridised with total DNA ^{3}H-cDNA, complementary to single-copy sequences, was prepared by removal of repeated sequences by hybridisation to C_0t 20–DNA–Sepharose as previously described[16]. The unhybridised cDNA (90% of the total) when reannealed to total DNA showed no repetitive sequences[16] The removal of the repetitive component of cDNA caused a marked depletion of the more abundant low complexity sequences. The remaining abundant sequences represented at most 20% of the single-copy cDNA[17]. Results are presented in integral form, that is as the percentage of total c.p.m. or absorbance having eluted up to each temperature. *a*, Thermal elution of rat DNA driver (○); rat ^{32}P-scDNA (□) and rat ^{3}H-cDNA (△); *b*, Thermal elution of mouse DNA driver (●), rat ^{32}P-scDNA (■); and rat ^{3}H-cDNA (▲); *c*, As panel *a* with the data normalised to 0% at 60 °C; *d*, As panel *b* with the data normalised to 0% at 60 °C.

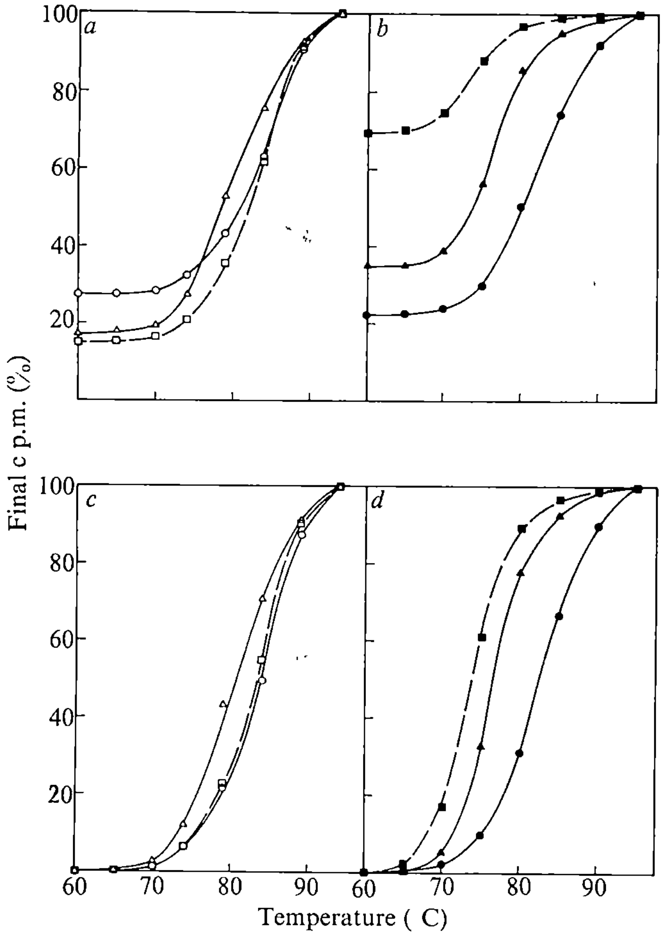


Fig. 2 Melting curves of hybrids of rat ^{3}H-cDNA and rat ^{32}P-scDNA to mouse and rat DNA incubated at 70 °C and assayed on hydroxylapatite. Conditions were as in Fig. 1 except that the incubation was at 70 °C.

DNA driver (450 nucleotides) Alternative interpretations include a base composition difference between cDNA and total DNA and low repetition frequency of a fraction of the "single-copy" cDNA

In contrast, both radioactive probes, when annealed to heterologous (mouse) DNA in identical conditions, have the ability to bind to hydroxylapatite in standard conditions (Fig. 1*b*), 82% for ^{3}H-cDNA and 67% for ^{32}P-scDNA as compared with 87% for both probes in the homologous reaction.

In addition, both rat cDNA and rat scDNA, when annealed to mouse DNA, elute at considerably reduced temperatures relative to homologous duplexes (Fig. 1*c* and *d*, and Table 1) Moreover, the ΔT_m of the rat scDNA–mouse DNA duplex (14 °C) is considerably greater than the ΔT_m of the rat cDNA–mouse DNA duplex (8 °C). This difference suggests that these two fractions of the genome are not identical; there exists considerably more mismatching and therefore evolutionary divergence between rats and mice in the total single-copy fraction of the genome than in the fraction of the genome complementary to the cDNA probe.

A ΔT_m of 14 °C represents a base substitution frequency of approximately 9% (ref. 11). Considering the moderate degree of stringency in this experiment, these data agree quite well with previously published reports which examined the relationship between rat and mouse single-copy DNA[6,7]. In contrast, it is relatively difficult to equate the 8 °C ΔT_m of rat cDNA–mouse DNA duplexes to a degree of base substitution because of the 3 °C ΔT_m of rat cDNA duplexes Without accounting for this homologous ΔT_m, the data suggest a 5% degree of mismatch between the rat and mouse sequences represented in cDNA. Subtracting this homologous ΔT_m yields a value of 4%. Consequently, we conclude that the rat cDNA–mouse DNA duplexes are between 4 and 5% mismatched.

Estimations of coding DNA

There are two methods of determining the proportion of rat scDNA which has the same T_m as the cDNA sequences. From the data in Fig. 1, it can be deduced that roughly 30% of the scDNA melting curve overlaps the cDNA melting curve and, therefore, has the same extent of divergence as mRNA.

The other method consists of establishing annealing conditions which allow only duplexes of little divergence to react. Incubation at elevated temperatures discriminates against molecules which are poorly matched and selects for molecules which have undergone little evolutionary change When the temperature of incubation is increased to 70 °C, there is little effect on the extent of homologous duplex formation or on the T_m of the hybrids formed (Fig. 2*a* and *c*) In contrast, the formation of heterologous duplex molecules with rat scDNA is markedly reduced. The fraction of rat scDNA which is retained by hydroxylapatite after incubation at 70 °C (Fig. 2*b*) is decreased to 30%; there is, however, only a small reduction in rat cDNA–mouse DNA duplex formation on increasing the temperature of incubation from 60–70 °C. These data are consistent with the original observation (Fig. 1) that there exists considerably more mismatching in rat scDNA–mouse DNA duplexes than in rat cDNA–mouse DNA duplexes.

Also consistent is the fact that the T_m of the rat scDNA–mouse DNA duplex molecules formed at 70 °C is considerably higher than the T_m of the duplex molecules formed at 60 °C, that is, the reduced fraction of duplexes which do form at 70 °C exhibit less mismatching than those formed in a less stringent criterion.

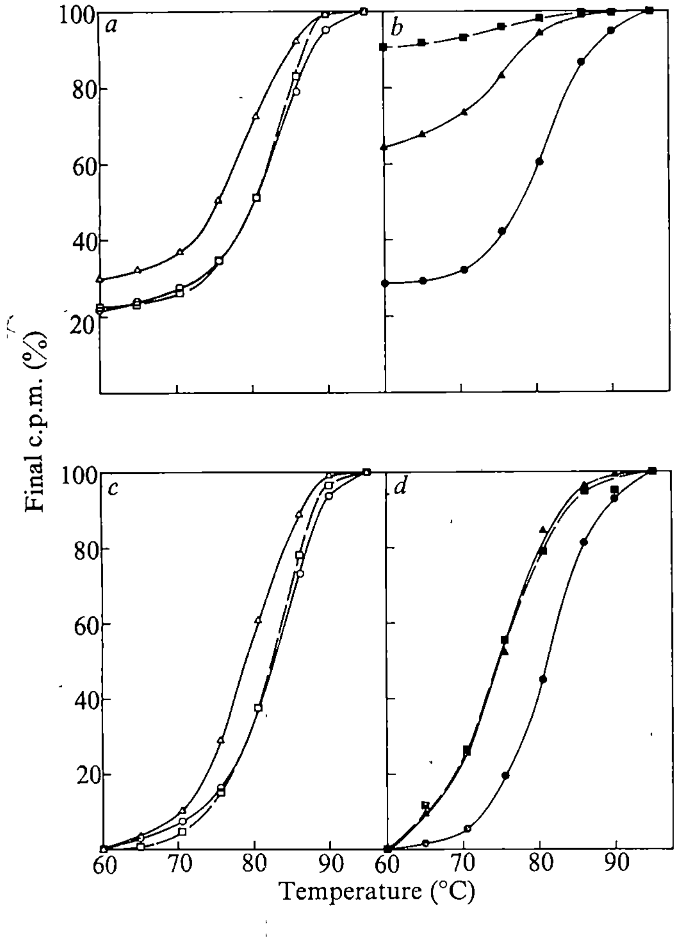
When the temperature of incubation is raised to 75 °C, the same trend continues. The homologous annealing between rat scDNA or rat cDNA and total rat DNA driver is only marginally affected by this increase in the temperature of incubation. The heterologous experiments are markedly affected. The extent of reaction is decreased, and the T_m of the duplexes is increased. At a temperature of incubation of 75 °C, only 9% of the rat scDNA forms duplex with mouse DNA. In identical conditions, 36% of the rat cDNA forms duplexes with mouse DNA (Fig. 3*b*). This represents a greater than sevenfold decrease in duplex formation for rat scDNA as compared with that observed at 60 °C but represents less than a 2.5-fold decrease for the rat cDNA. Moreover, at this stringent criterion, the T_m of the rat cDNA–mouse DNA duplex structures is identical to the T_m of the rat cDNA–mouse DNA duplexes (Fig. 3*d*).

It is possible that only a fraction of the nucleotides that bind to hydroxylapatite after incubation at 75 °C are in duplex form. This possibility predicts that only a fraction of each cDNA molecule and, therefore, only a fraction of each rat mRNA molecule from which it is synthesised, is relatively homologous to mouse DNA. To examine this possibility, the structures formed after incubation at 75 °C were assayed with S1 single-stranded nuclease[12] (Fig. 4). In all cases the extent of duplex formation is similar to the identical experiment as assayed with hydroxylapatite (Fig. 3). In particular, after incubation with mouse DNA, only 7% of the rat scDNA is nuclease resistant. But, 35% of the rat cDNA is nuclease resistant. In addition, the T_ms of these duplex structures are identical, as are the T_ms when assayed with hydroxylapatite (Fig 3*d*) These data confirm our interpretation that the majority of single-copy DNA diverges more rapidly than mRNA sequences.

The distribution of divergence in the two populations of molecules is very different. Total single-copy DNA has a broader spectrum of sequence divergence than protein-coding sequences. The upper limit of its distribution overlaps the tighter distribution of the cDNA sequences though the distribution is weighted toward diverged sequences. Conditions which select for sequences with the same distribution will reduce the formation of heterologous rat scDNA duplexes more than the heterologous cDNA duplexes If the extent of scDNA duplex formation at 75 °C is normalised to the cDNA duplex formation at 75 °C, the proportion of the scDNA sequences with an identical T_m is 18%. We feel that these data indicate that at most 20–30% of the scDNA of the rat has the same degree of divergence observed for mRNA sequences. There should be some single-copy DNA with greater than average sequence conservation due to stochastic processes in mutation; in addition, there might be functions other than protein synthesis for which conservation of sequences is important. Consequently, this number is very much an upper limit for the potential protein-coding DNA.

It is crucial to ascertain to what extent the properties of this cDNA probe are representative of the sequence properties of general messenger RNA. When hybridised in RNA excess to its template poly(A)-containing RNA[5], the cDNA is rendered double stranded with kinetics which suggest that there are about 6,000–7,000 different mRNA sequences[14]. These data also show that there is no very low complexity component in the mRNA which may render the cDNA probe unrepresentative; and that the more abundant sequences in the mRNA represent at most 20% of the cDNA probe and cannot thus be responsible for the extensive cross hybridisation observed. RNA excess hybridisation, however, measures the complexity of the RNA population and not the radioactive tracer; it is therefore possible that the cDNA probe is of relatively low complexity and homologous to only a fraction of the poly(A)-containing sequences. Verma *et al.*[15] have measured the ability of cDNA to render its template, *Dictyostelium* poly(A)-containing RNA, double stranded in conditions of cDNA excess. This cDNA population seemed to contain all of the sequences present in the poly(A)-containing RNA population. Until a comparable experiment has been performed in this system, the complexity of this cDNA probe is somewhat uncertain. There is, however, little information to suggest selective transcription of specific poly(A)-containing sequences and it is therefore likely that this cDNA probe is a complex mixture of 6,000–7,000 different sequences.

Fig. 3 Melting curves of reassociations of rat ^{3}H-cDNA and rat ^{32}P-scDNA to mouse and rat DNA incubated at 75 °C and assayed on hydroxylapatite. Conditions were as in Fig. 1 except that the incubation was at 75 °C



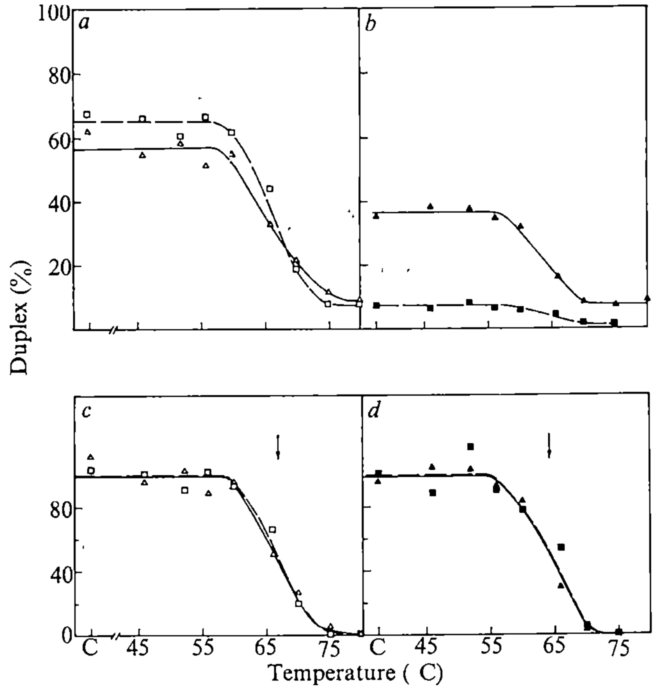


Fig. 4 Melting curves of reassociations of rat ^{3}H-cDNA and ^{32}P-scDNA to mouse and rat DNA incubated at 75 °C and assayed with S1 nuclease. Reassociations were carried out in 0 24 M PB at 75 °C to a C_0t of 10,000 Mixtures were diluted 20-fold with ice cold water (to a concentration of 0.012 M PB) and divided into 12 samples The samples were heated from 45 to 85 °C in 5° increments. After 5 min at each temperature, one sample was removed, chilled, and treated at 50 °C for 40 min with S1 as previously described[3]. *a*, Rat ^{3}H-cDNA (△) and rat ^{32}P-scDNA (□) reassociated to rat DNA; *b*, ^{3}H-cDNA (▲) and rat ^{32}P-scDNA (■) reassociated to mouse DNA; *c*, As panel *a* with results expressed as % of total counts hybridised; *d*, As panel *b* with results expressed as % of total counts hybridised. The arrows in panels *c* and *d* indicate the T_m.

It is also possible that these rat myoblast poly(A)-containing sequences represent only a limited fraction of the total genetic information required during the lifetime of the entire organism and therefore, our conclusions are limited to such a set of sequences. In addition, there might be some mRNA lacking poly(A) which would be undetected in the present study[16]. Nevertheless, we feel that this number is large enough to represent an average population of mRNA, and although proteins evolve at vastly different rates, it is expected that a population of 7,000 different proteins will reflect the average properties of the total complement of proteins of which it is a subset

The cDNA used in this experiment was, on average, 370 nucleotides long, and should represent approximately 18% of the length of messenger RNA molecules[5], although it undoubtedly represents the sequence at the 3′ end of the messenger molecules more frequently since it is primed by $(pT)_{10}$ from the poly(A)-containing end. Work by Proudfoot and Brownlee[17], indicates that there exists an identical poly(A)-proximal 3′ sequence in both mouse immunoglobulin mRNA and rabbit haemoglobin mRNA. In fact, this 3′ sequence is too small to be detectable by hybridisation, which is consistent with the single-copy nature of the cDNA probe. Significant homology between the 3′ untranslated regions of different mRNA sequences is therefore unlikely. Nevertheless, sufficient homology might exist between the 3′ untranslated portion of the mRNA molecule which codes for the same protein in both rats and mice to influence the experiments presented here. Preliminary experiments to investigate the thermal stability of heterologous mRNA–DNA duplexes argue against this possibility (K.S.G., M.S.C. and M R , unpublished). In this case, the rat mRNA–mouse DNA duplexes have a T_m only 2 °C below the T_m of the homologous duplex structures. This is the same result obtained in experiments on haemoglobin mRNA and is consistent with the interpretation presented above[8]. These experiments also suggest that the increased ΔT_m observed with rat myoblast cDNA (5–8 °C) as compared with rat myoblast mRNA (2 °C) may be due to the presence of an increased representation of 3′ untranslated regions of these mRNA sequences in the cDNA.

These experiments deal exclusively with the evolutionary distance between rats and mice, and, therefore, with evolutionary events which have occurred in these two rodent species during the past 5–10 Myr It is conceivable, although unlikely, that such conclusions are uniquely applicable to rodents, recent evolutionary events, or both. Nevertheless, these results suggest that most of the DNA in mammalian genomes is not involved in translation. Those sequences which are translated comprise at most 30% of the unique nucleotide sequences of the genome and are relatively conserved in nucleotide sequence. The remaining sequences accept nucleotide substitutions more readily, as indicated by the greater divergence of scDNA as compared with cDNA.

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letters to nature

Size distribution of cosmic gamma-ray bursts

We report on the combined results of three balloon-borne searches for small cosmic γ-ray bursts, carried out by the Imperial College Space Physics Groups. We suggest that our results are incompatible with the extragalactic source postulated for the large events previously seen by satellites. No decisive directional information has been obtained from the timing measurements, carried out by the Vela satellites on the large (10–100 photon $\mathrm{cm^{-2}\,s^{-1}}$(> 100 keV)) events, although a weak correlation with the local spiral arm exists[1].

Description of the apparatus and results of our first flight have been reported[2], but a significant increase in viewing time has been achieved. Briefly, a flight of 4.5 h at 5 mbar was achieved from Aire Sur L'Adour on September 12, 1974, with a detector consisting of 10^4 cm^2 of plastic scintillator, 5 cm thick and sensitive to γ rays in the range 15 keV to 1 MeV. One unconfirmed event was seen of size 1.2×10^{-7} erg cm^{-2} and duration 5 s. Two further flights from Gap (France) were made on June 11 and 21, 1975, with the same plastic scintillator, split into two slabs, each inclined at 30° to the horizontal to gain some directional information. In 9.0 h at an altitude of less than 5 mbar no candidate events were detected. The event search was carried out using various integration times up to 8 s. It is interesting that of the seven large bursts seen by the Vela network whose time profiles are well determined from other satellite data, six produced most of their energy within 8 s of their peak intensity.

If the sources of γ-ray bursts have a well behaved distribution about a mean strength, and are uniformly distributed in space, it is a consequence of the inverse square law that the observed burst size S in erg cm^{-2} integrated over the event duration and the rate of events $N(S) > S$ are related by $N(S) \propto S^{-\alpha}$ where $\alpha = 3/2$. Alternatively, if the sources are confined to an infinite thin sheet approximating the shape of the galactic disk then $\alpha = 1$. Although this does not require sources to have a uniform intrinsic luminosity and time behaviour, it does depend on the width of the intrinsic source distribution being relatively small compared with the range of sizes measured experimentally, and also on the event duration being small compared with the occurrence rate. It is important to compare the satellite and balloon rates, spanning nearly three decades in size to investigate if either of these predicted distributions fits the data.

A total of 23 Vela bursts, as confirmed by multi-satellite recordings, have accurately known sizes S. Strong *et al.*[1] have already plotted a size spectrum for these events in terms of erg cm^{-2} and shown a fall off from the $-3/2$ low at low intensities. This, however, may be due to a lack of sensitivity in the triggering system used in their experiment[3]. Figure 1 shows the size spectrum as plotted by Strong together with the Imperial College data, adjusted to a rate from 4π sr and for the same period as used by Strong. In plotting the balloon data, we assume the standard $\exp(-E/E_0)$ spectrum with $E_0 = 150$ keV. Note that if the '3/2' power law holds we are

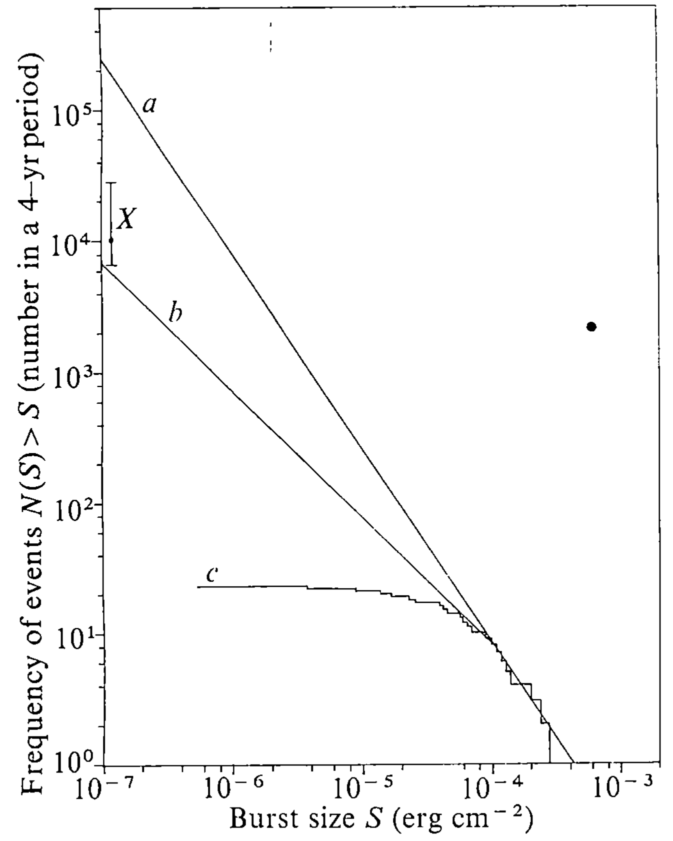

Fig. 1 Plot of burst size (S) against frequency. Our point (X) is marked with $\pm 1\sigma$ error bars calculated from a Poisson distribution. The smooth curves (a and b) give the theoretical $S^{-3/2}$ and S^{-1} distributions extrapolated from Vela results (curve c)

entitled to normalise at any point on the experimental Vela spectrum. The balloon point can be seen to lie close to the S^{-1} extrapolation of the Vela point and the probability of its being a statistical fluctuation from the $S^{-3/2}$ law is negligible. The magnitude of random errors in the size determination from the Vela point is given by the departure of the points from the smooth curve, which approximates the true burst occurrence rate. Any systematic errors in the satellite data, such as missing part of a burst due to the triggering system, will cause the Vela size spectrum to move towards higher sizes, thereby increasing the discrepancy from the '3/2' power law. Similarly, sources of error in the Imperial College result, namely the unconfirmed nature of the event, uncertainties in the angular window of the detector and a possible lower effective energy threshold, can only move the point further from the $-3/2$ line. We have also investigated the size spectra from the two detector types as a function of photon intensity, defining an effective pulse time and mean height, and get essentially the same discrepancy from the $-3/2$ power law. Thus we believe that the conclusion is insensitive to the exact manner in which source strength is defined and to the assumption concerning the spectral form of the candidate balloon event. We can therefore be reasonably confident in excluding a uniform source distribution throughout space (with a relatively narrow intrinsic source distribution), but cannot confirm a galactic disk distribution. Indeed, if the balloon point is not genuine, then the only real events may be large local happenings, all of which we see. One possibility that neither the balloon nor Vela data can adequately investigate, is that an appreciable fraction of burst events occur over time scales larger than 8 s, with slow rise and fall times, and a small peak intensity–time scale ratio compared with those events presently known[3]. It is possible to imagine an absolute size dependence for this ratio which would invalidate any simple conclusions concerning deviations from a $-3/2$ power law. A more implausible alternative may be that we are seeing most events from a universal distribution with a rather flat size distribution extending over nearly four orders of magnitude.

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Cosmic ray streaming

TAKING the confinement mechanism for cosmic rays in the Galaxy to be resonant scattering by Alfvén or hydromagnetic waves generated by the streaming of the cosmic rays themselves[1,2], I shall show that the abundant low energy cosmic rays can generate, indirectly, enough Alfvén waves of long wavelength to scatter cosmic rays of considerably higher energy. Cosmic rays of energy up to around 1,000 GeV may thus be confined within the Galaxy more efficiently than has previously been supposed.

In highly ionised regions of interstellar space, it is thought that the Alfvén waves so generated, which propagate 'forwards' along the ambient magnetic field $\mathbf{B}_0$ in the direction of decreasing cosmic ray density, are removed by a cascade of stimulated decay processes involving reversal of the Alfvén wave direction and emission of sound waves[3]. Schematically,

$$\mathrm{A}_+ \rightarrow \mathrm{A}_- + \mathrm{S} \text{ followed by } \mathrm{A}_- \rightarrow \mathrm{A}_+ + \mathrm{S}^*,$$

where $+(-)$ refers to a forward (backward) Alfvén photon. At each wave–wave interaction, the Alfvén photon loses some energy to a (compressional) sound wave, which can be damped by thermal conductivity. (Note that the resonant wavelengths are sufficiently long, for cosmic rays of interest, that the plasma is collisional.)

Full details of the analysis are being presented elsewhere[4–6], but as long as the sound waves remain heavily damped the steady-state equations governing this cascade are

$$p\frac{\partial \mathscr{I}}{\partial p} = \frac{\alpha_0 \pi v_{\mathrm{A}} p^5}{3U_{\mathrm{M}}} \frac{\partial f}{\partial p} \tag{1}$$

$$p\frac{\partial \mathscr{A}}{\partial p} = \frac{-4\alpha_0 p^5 v f}{3m\Omega_0 U_{\mathrm{M}} \mathscr{I}^2 L} \tag{2}$$

(equations 11 and 12 of ref. 6). Here $\mathscr{I} = \mathscr{I}_+ + \mathscr{I}_- =$ total Alfvén wave intensity per unit log bandwidth (relative to $U_{\mathrm{M}} = B_0^2/2\mu_0$), $\mathscr{A} = (\mathscr{I}_+ - \mathscr{I}_-)/(\mathscr{I}_+ + \mathscr{I}_-)$ is the wave asymmetry, α_0 is a numerical constant relating wavenumber k and particle momentum p in an approximate resonance condition $\alpha_0 kp = m\Omega_0$ (for the galactic particle spectrum, $\alpha_0 = 0.41$) and $L = fB_0/|\mathbf{B}_0 \cdot \nabla f|$ is the cosmic ray scale length along field lines. For the escape of cosmic rays from the Galaxy, we shall assume that L is at least approximately independent of energy. In deriving (1) and (2) the wave–wave interaction formulae[3] have been coupled with the linear growth or decay of waves due to cosmic rays, expressed in terms of the gradients of the cosmic ray distribution function f.

These two equations determine the Alfvén wave intensities appropriate to a given spectrum of cosmic rays. They do not necessarily imply that the intensities fall off with increasing

p, as f does. Being differential equations, they should be solved with the aid of two boundary conditions. One such boundary condition is that $\mathscr{I}_-$ should vanish ($\mathscr{A} = 1$) at the 'top' of the cascade (largest k, smallest p). This is because the photon flux down the cascade (which varies as the product $\mathscr{I}_+\mathscr{I}_-$ because it arises from the decay of A_+ stimulated by A_-) must vanish at that k above which there is no further input of photons.

A second boundary condition can be set by the breakdown of the cascade equations at small k (large p). When the wavelengths become large, the sound waves are no longer heavily damped and their intensity rises. A highly asymmetric distribution of Alfvén waves ($\mathscr{A} \simeq 1$) cannot be maintained in the presence of an appreciable level of sound waves, and in fact the cascade equations break down when the dimensionless sound damping coefficient $\mathscr{D} = 4\sigma_S/\pi k v_A \mathscr{I}$ falls to the value $v_A{}^2/v_S{}^2$ (ref. 5). The asymmetry $\mathscr{A}$ is unable to remain close to unity for yet smaller values of k.

On the other hand, $\mathscr{A}$ cannot decrease below (say) 1/2 before the cascade equations break down, because the ever faster rate of decrease of $\mathscr{A}$ (see equation 2 with $f \propto p^{-4.6}$) as k diminishes would soon give a solution with $\mathscr{A} < -1$, which is clearly impossible. Thus $\mathscr{A}$ must pass through some value like 1/2 just when $\mathscr{D}$ falls to $v_A{}^2/v_S{}^2$. In fact, the solutions are fairly insensitive to this condition. The two boundary conditions are

$$\mathscr{A} = 1 \text{ at } p = p_{\min} \tag{3}$$

$$\mathscr{A} \simeq 1/2 \text{ when } \mathscr{D} = v_A{}^2/v_S{}^2 \tag{4}$$

Taking as typical parameters for the ionised interstellar medium $|\mathbf{B}_0| = 3$ μgauss, $n_e = 10^5$ m^{-3}, $T = 7{,}000$ K and taking $L = 500$ pc, the solution of (1) and (2) under (3) and (4) has the total wave intensity dominated by its constant of integration

$$\mathscr{I} \simeq \mathscr{I}_0 = 4\times10^{-4} \tag{5}$$

The corresponding asymmetry obeys $1-\mathscr{A} \ll 1$ until the bottom of the cascade is approached. (The way in which the results scale with interstellar parameters is to be found in ref. 6.) Even at the lowest particle energies of ~ 1 GeV, $\mathscr{I}_0$ exceeds the $O(p^4 v_A f/U_M)$ term directly contributed to $\mathscr{I}$ by the right of equation (1). Formally, this solution holds up to a particle energy

$$(p/mc)_{\max} = 7\times10^6, \text{ or } E = 7\times10^6 \text{ GeV} \tag{6}$$

determined by equation (4). It is, however, possible that general interstellar turbulence may cause even more energy to be given to those waves which resonate with such high energy particles[7,8].

The relatively large wave energy (5) enables cosmic rays to be confined very efficiently, with only a small diffusion coefficient. The waves are, however, nearly all forward waves. The net effect of diffusion through a system of waves moving in one direction at the Alfvén speed is to give particles of a given energy a net anisotropy

$$a = (1.5v_A + (D_{\|}/L))/v$$

where 1.5 is the Compton–Getting factor and the diffusion coefficient is $D_{\|} = 4pv/3\pi m\Omega_0\mathscr{I}_0$. Thus the predicted anisotropy at a typical location within the galactic disk is, numerically

$$a = (1.0 + (E/180 \text{ GeV}))\times10^{-4} \text{ for } L = 500 \text{ pc} \tag{7}$$

The corresponding anisotropy for a shorter assumed scale length is

$$a = (1.0 + (E/100 \text{ GeV}))\times10^{-4} \text{ for } L = 200 \text{ pc} \tag{8}$$

Even 1,000 GeV particles have an anisotropy less than 0.1%, in keeping with observational constraints up to that energy.

To conclude, the intensity of self-generated Alfvén waves may be much higher than the $O(p^4 v_A f/U_M)$ which one would suppose for power law solutions of the wave equations, in particular equation (1). Instead, the constant of integration dominates, and this gives a 'flat' spectrum having equal energy in each octave of waves. These high intensity waves give good confinement of cosmic rays having $E \lesssim 1{,}000$ GeV. Above this energy, though, it may be necessary to appeal to an interstellar turbulence spectrum for a source of hydromagnetic waves.

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Third speed-up of the Crab pulsar

OPTICAL timing of the pulsar in the Crab nebula revealed a further speed-up by $\Delta\nu = 0.097$ periods d^{-1} = $3.7\times10^{-8}\,\nu$, on 1975 February 4 (JD 2442447.9). The initial jump in pulse frequency was at least three times larger than that reported[1] for the first glitch, and allows a better estimate of the glitch parameters.

Arrival times of optical pulses from the Crab pulsar were measured at the 60-cm refractor of the Hamburg–Bergedorf Observatory. The typical error for one night is about 10 μs = 0.0003 periods relative to Loran-C Sylt signals. The barycentric arrival times were computed using a program by J. M. Rankins[2] and an ephemeris tape prepared by the MIT Lincoln Laboratory[3].

Figure 1 shows time residuals (observations−calculations) from a cubic fit to the barycentric arrival times before the glitch according to:

$$O-C = \varphi_0 + (t-t_0)\nu_0 + (t-t_0)^2\nu_0/2 + (t-t_0)^3\nu_0/6 - N$$

Only φ_0, ν_0, and ν_0 have been fitted: ν_0 was fixed, because short time spans usually yield meaningless values of ν_0 (see ref. 4 and Fig. 1 of ref. 5). The value adopted, $\nu_0 = 7.95\times10^{-6}$ d^{-3}

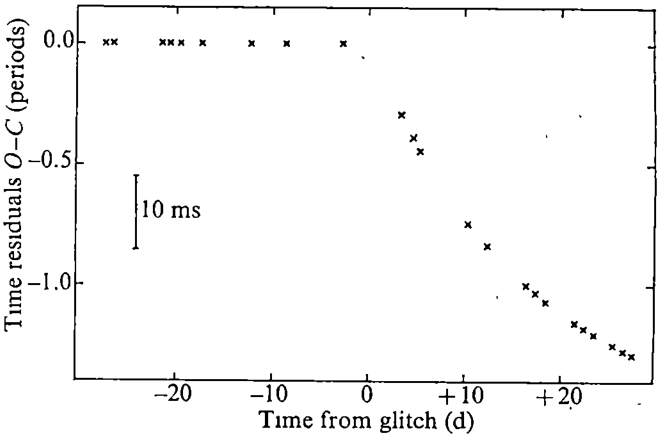

Fig. 1 Observed pulse arrival times minus arrival times computed from cubic fit to the data before the third glitch.

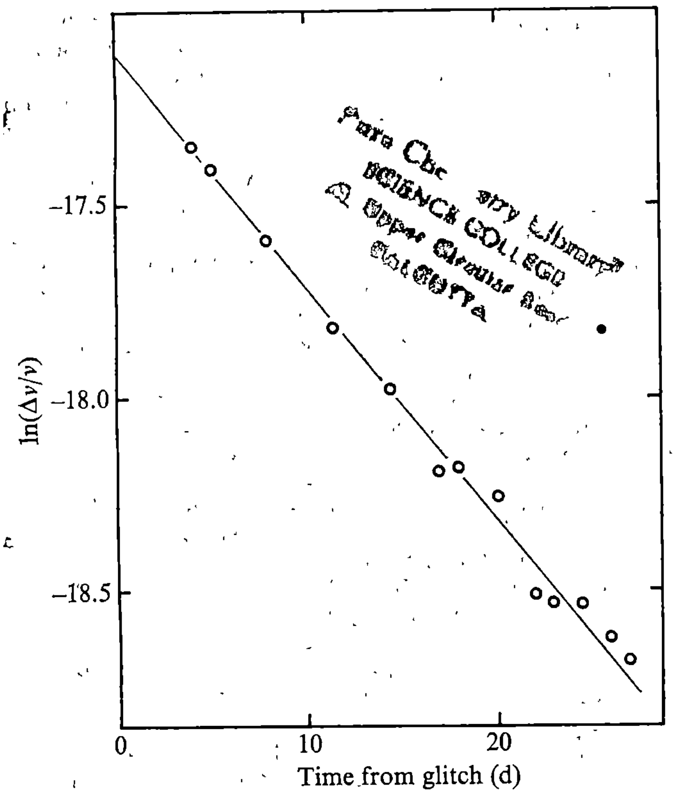

Fig. 2 Natural logarithms of excessive pulse frequency.

corresponds to a braking index n (≡ $\nu\nu/\nu^2$ =) 2.523, which is very close to the mean value[6] over the five years following the first speed-up. The observational errors and the deviations from the fit are too small be be seen on the scale of Fig. 1. A sharp increase in the pulse frequency, resulting in an initial phase shift of 0.1 period d^{-1} appears after February 4. The frequency shift slowly decays, approximately following an exponential law.

In Fig. 2 the natural logarithms of the frequency differences $\Delta\nu$ ($\nu_O-\nu_C$) from the cubic fit are plotted. Here $\Delta\nu$ has been taken as the ratio of the differences in $O-C$ divided by the differences in time for each pair of adjacent observations. The error bars include observational errors only. A straight line drawn through the points of Fig. 2 yields a relaxation time of 17 d for the frequency jump.

A two-component model, proposed by Ruderman[7] and Baym *et al.*[8] has been applied to the first glitch of the Crab pulsar by Boynton *et al.*[1]. According to this model the residuals $O-C$ from a cubic fit are

$$O-C = -\Delta\nu_0\{Q\tau(1-\exp[-(t-t_g)/\tau])+(1-Q)(t-t_g)\}$$

where $\Delta\nu_0$ is the initial frequency jump, τ the relaxation time, t_g the time when the glitch occurred, and Q a structure parameter equal to the ratio of the moments of inertia of the superfluid component to the moment of inertia of the whole star.

The parameters of the three glitches known at present are compiled in Table 1, where data for the first glitch are from ref. 1. The errors are twice the formal errors of the fit. The second[9] speed-up was too small to give definite values of Q and τ at the same time, therefore Q has been fixed and the post-glitch span has been restricted to 14 d; longer fits with free Q yield $Q\sim 0.65$ and $\tau\sim 5$ d. It might be possible to gain useful information by combining this small glitch with events of similar size to reduce the frequency noise. At the moment it is not clear what causes the discrepancy between the relaxation times of the first and the third event, but two sources of errors appear in the pre-glitch data of ref. 1: first the large residuals displayed during the summer months—possibly the consequence of an earlier large glitch—make it difficult to estimate ν and ν from a longer interval, and second, the large gaps in the two shortest fits make it impossible to determine ν and ν reliably. Keeping in mind these difficulties it seems unjustified at present to propose essentially different relaxation times for the first and the third glitch.

Because of the frequent small jumps in period, all parameters derived from fits of timing observations depend on the length of the fit, the weighting, and the distribution of the data points. In the data of the third glitch, however, this effect is not serious for short spans. Even the shortest fit, extending to 12.5 d after the glitch only, yields parameters which agree well with those in Table 1, namely $\Delta\nu_0 = 0.097\pm0.006$ d^{-1}, $Q = 1.04\pm0.56$, t_g = JD 2442447.96±0.15, and $\tau = 16.5\pm14$ d (formal mean errors), where the large errors in Q and τ may be explained by the high correlation between these two parameters. If we go to larger intervals, the parameters stay within the limits of Table 1 when two further points, −42 and +33 d from the glitch are included. If we include points at 47 d and more after the glitch, Q exceeds 1 and τ becomes greater than 20 d. It is possible, however, to keep Q and τ within the limits of Table 1 if we change ν by ~ 20%, which is within the range experienced when fitting polynomials to spans of comparable length during 'quiet' periods.

Table 1 Parameters for three Crab pulsar glitches

Date	1969 September 29	1971 October 26	1975 February 4
Julian day −2440000.5	0493.8±0.6	1,250.3±0.3	2,447.4±0.1
$\Delta\nu_0$ (d^{-1})	0.025±0.009	0.0052±0.0006	0.097±0.002
$\Delta\nu_0/\nu$ (×10^9)	9.5±3.5	2.0±0.2	37.2±0.8
τ (d)	4.1±2.2	15.2±4.0	15.5±1.2
Q	0.93±0.05	0.96 (fixed)	0.96±0.03
Span of fit			
pre-glitch (d)	37	22	27
post-glitch (d)	25	17	28

So, at present there is no need to assume a significant deviation from the two-component model. It may be hoped that a somewhat better value for Q will be obtained from the better coverage of this last event, especially from timing data of the month preceeding and the two weeks following the range of Fig. 1.

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High temperature condensates among meteors

HARVEY has reported[1] the spectra of two meteors which exhibited no lines of iron or sodium, but which did show lines of calcium and magnesium as well as of aluminium and silicon. Meteors characterised by this type of spectrum constitute about 1% of the 500 meteors observed by the NASA-LRC Faint Meteor Spectra Patrol[2]. Harvey recognised that the spectra indicated chemically differentiated material, and suggested that the

Table 1 Average Na and Fe contents of meteorites* and the lunar surface

Sample	Class	Na(10^{-6}g g^{-1})	Fe (%wt)
Carbonaceous chondrites	C1	5,100	18.4
	C2	3,800	21.9
	C3	3,500	25.2
Ordinary chondrites	H	5,700	27.6
	L	6,500	21.8
	LL	6,600	20.0
Enstatite chondrites	E4	8,200	33.0
	E5,6	5,200	25.5
Ca-poor achondrites	Aubrites	3,500	1.0
	Ureilites	300	14.4
	Diogenites	120 (ref. 13)	13.5
	Chassignite		20.6
Ca-rich achondrites	Eucrites	2,800	13.9
	Howardites	1,900	14.4
	Nakhlites	3,300	16.6
	Shergottite	11,000	
	Angrite		7.5
Lunar surface		1,430–5,000 (ref. 14)	6.5 (ref. 15)

*Except as noted all data are from ref. 12.

meteoroids were composed of enstatite achondritic meteorites (aubrites) to explain their lack of iron.

Obtaining elemental abundances from meteor spectra is difficult. Since the Na D lines are, however, among the most intense of the persistent lines of the elements, the absence of Na from the spectra merits consideration. In Table 1 are listed Na and Fe compositions of various well studied Solar System materials, including aubrites. It can be seen that none simultaneously shows low iron with low Na. The Na content of aubrites, which is similar to that of carbonaceous chondrites, is not unusually low. Observations[2] and laboratory experiments[3] indicate that most meteors are produced from undifferentiated material having non-atmophile elements present in amounts similar to those in carbonaceous chondritic meteorites. Sodium is always a feature of their spectra. Thus, it seems necessary to consider possible parent materials other than aubrites to produce the observed iron- and sodium-free meteors.

In searching for another source, we started with the minerals thought to be of cosmological interest which contain both Ca and Mg but neither Fe nor Na, namely, melilite, ($Ca_2Al_2SiO_7$–$Ca_2MgSi_2O_7$) and diopside ($CaMgSi_2O_3$). These minerals are among those predicted by Grossman[4] to be early (or refractory element) condensates of the primitive solar nebula, that is, chemical compounds which would condense from a cooling gas of solar composition at a total pressure of 10^{-3} bar and temperatures exceeding 1,400 K. This suite of rather unusual minerals has received wide attention because the minerals have been identified in light-coloured, millimetre-sized polymineral inclusions present in carbonaceous chondrites (reviewed in ref. 4). It should be noted that almost the entire suite can be formed from Mg, Ca, Al and Si, the two exceptions being the Fe–Ni alloy and Ti-bearing perovskite ($CaTiO_6$) Metallic Ni–Fe is not found in association with the other members of the suite in carbonaceous chondrites[5], so the absence of Fe from the meteors is not surprising. The other minerals in the suite are corundum (Al_2O_3), spinel ($MgAl_2O_4$), and forsterite (Mg_2SiO_4). By reason of its refractory nature, Ti should also be present in these meteoroids. It might, however, not have been detected in the meteor spectra because of the experimental difficulties reported by Harvey[3] for other refractory elements, and because of its lower abundance, 3×10^{-3} times the cosmic abundance of Al.

There is at least one problem in identifying the refractory inclusions found in meteorites with the Mg, Ca-rich meteors, namely, some inclusions in the carbonaceous chondrite Allende have rather high Na contents (up to 25,000 p.p.m.[6]). The Na seems, however, to be located in the inclusions in separate mineral phases, such as sodalite ($Na_8(Al_6Si_6O_{24})Cl_2$) and nepheline ($NaAlSiO_4$) and there is evidence that the alkali-bearing minerals were formed by chemical reactions which occurred after the inclusions were emplaced in the carbonaceous chondritic matrix[4,6–8]. It should be mentioned that the refractory minerals, as opposed to the alkali-bearing minerals, in the inclusions are free of Na and Fe[9].

Because of the ubiquity of Fe and Na in planetary materials, it is necessary to eliminate all known types of meteorites from consideration. Since most stony meteorites, especially the chondrites, are complex mixtures of materials, which may have had differing origins and histories, it is reasonable to suppose that the components of meteorites such as the refractory inclusions, may be found individually in space. The refractory inclusions found in carbonaceous chondrites have in all cases been small objects typically less than a centimetre in diameter. Their small size and undistinguished appearance may explain why none has been found as an individual meteorite on Earth.

On the basis of spectral indicators, Harvey[1] assigned the Ca, Mg-rich meteors high velocities ($V > 40$ km s^{-1}) and, hence, high inclination and/or retrograde orbits. Such orbits are generally associated with comets, although some asteroids are also found in highly inclined orbits. Comets are believed to be the most volatile-rich objects entering the inner Solar System, whereas the refractory minerals are the least volatile substances known. Such a vast compositional difference would seem to preclude a genetic relationship, other than mixing at low temperatures, between the two, in spite of their similar orbits.

The interesting possibility arises that these meteoroids could form a reservoir for the so-called 'lost' elements: Ca, Mg, Al, Ti, the lanthanides, and other refractory elements which are depleted in ordinary and enstatite chondrites relative to cosmic abundances[11]. This suggestion would seem to require that early condensates from the solar nebula were separated from later condensates by some process which resulted in long period, comet-like orbits for the refractory materials. More studies are needed to determine whether or not high entry velocities are indeed characteristic of these meteors. Further compositional data on meteors should help to clarify the nature of the interrelationships among individual meteoritic components, asteroids and comets.

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Detection of a potassium cloud near Io

JUPITER's innermost Galilean satellite Io is surrounded by an extensive cloud of sodium and hydrogen[1,2]. The hydrogen is observed to extend along a segment of Io's orbit but the sodium cloud envelopes the entire Jovian system, extending tens of arc min from Jupiter. It is brightest, however, near Io and in its orbital plane. Sputtering by

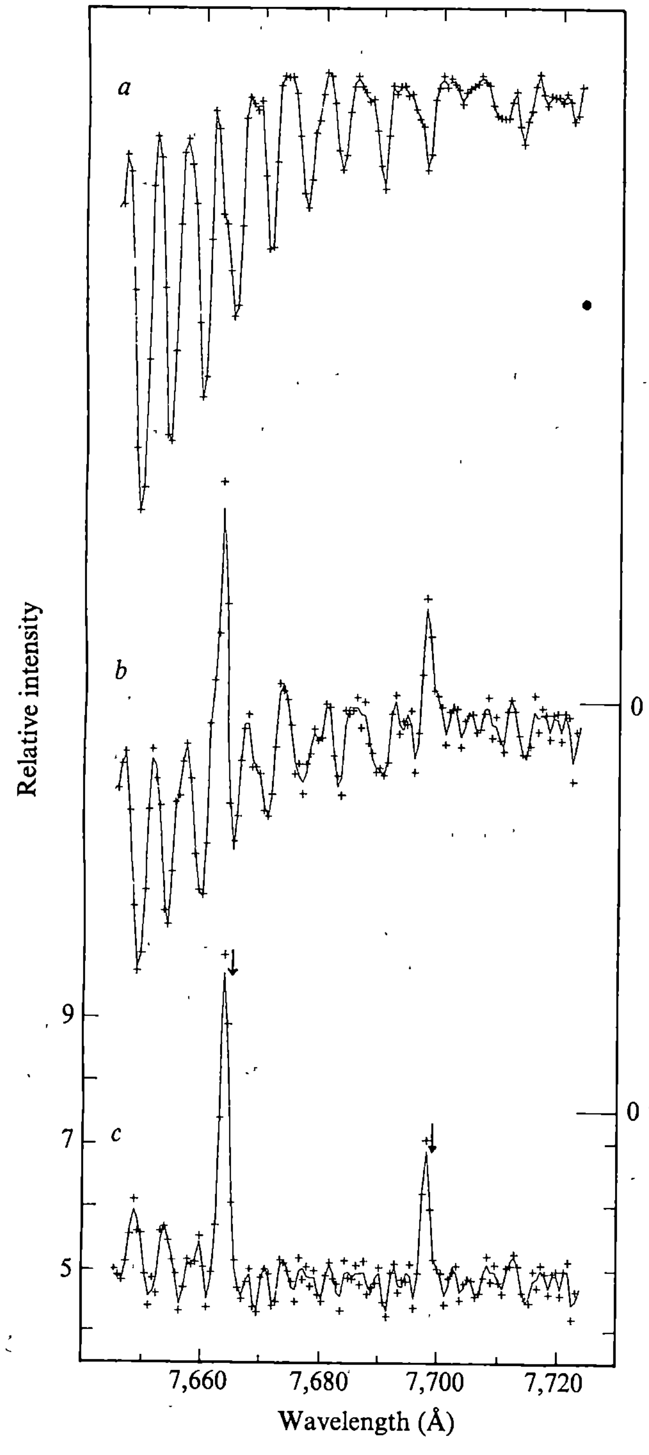

Fig. 1 Potassium emission in Io's cloud. These spectra were taken on September 5, 1975 using the coude scanner of the 107-inch telescope at the McDonald Observatory. The resolution is 1.3 Å. The original data points are shown superposed on the slightly smoothed spectra. *a*, Spectrum of Io in the region of the potassium resonance lines showing absorption from the strong telluric O_2 band lying between 7,594 Å and 7,700 Å. *b*, Spectrum of Io's cloud 7½″ east of Io showing the potassium lines at 7,665 Å and 7,699 Å in emission. Io was east of Jupiter at this time, with an orbital phase angle of 64° from superior geocentric conjunction. The spectrographic parameters were the same as those for *a* except the entrance slit measured 3.0″×22.5″ instead of 3.0″×4.2″. Io's diameter at this time was 1.21″. *c*, the ratio spectrum. The telluric absorption is nearly cancelled out. The arrows mark the wavelengths of these lines in the Earth's rest frame. The emission lines are blueshifted 1.1 ± 0.3 channels, a result consistent with the 1.3 channels expected from Io's Doppler shift of −0.84 Å relative to the Earth.

charged particles impacting the surface of Io has been the most plausible hypothesis for supplying the sodium[3]. The hydrogen may result from neutralisation of protons trapped in the Jovian magnetosphere when they impact on Io's surface[4]. Resonant scattering seems to be the dominant emission mechanism[5,6].

Sputtering suggests that other elements in Io's surface layer should also populate the cloud. Various searches for such elements, however, have not proved successful[1,7,8]. Even when Io's disk was excluded from the spectrograph slit, no emission other than from sodium was obvious[9]. I report the first detection of potassium in Io's cloud. Its presence with hydrogen and sodium, along with the absence of more abundant elements such as calcium, might be explained by a rough surface microstructure for Io and a process for multiple impacts of the sputtered products with the surface.

Figure 1*a* and *b* shows a spectrum of Io and of a point 7½″ east of Io in the telluric oxygen band at 7,600 Å. Figure 1*c* shows the ratio spectrum and the emission lines free of the telluric absorption. The emission features lie at the wavelengths of the potassium resonance lines 7,665 Å and 7,699 Å in Io's Doppler shifted reference frame. The relative intensity of the two lines is compatible with that of their oscillator strengths, as expected for an optically thin medium. The absolute intensity is not great enough for these lines to be visible when Io's bright disk lies in the spectrographic slit.

Spectra taken 1° north of Io immediately afterwards showed no hint of potassium emisson. A spectrum taken 15″ east of Io showed the emission at about half the strength. A final spectrum at 7½″ east of Io, 1 h after that given in Fig. 1*b* showed the emission strength to be essentially unchanged. Thus, the emission is associated with Io and lies, like sodium, in an extensive cloud about Io.

Since only elements with unit valence have been detected in Io's cloud, there is a process or condition which favours these elements relative to the cosmically more abundant ones. One interpretation is that Io's surface is enriched in the observed elements relative to solar, lunar or meteoritic abundances. Fanale *et al.*[10] have advanced an evaporite salt hypothesis which has been used to explain this enrichment. They suggest Io's surface is rich in the salt residues of solutions which migrated to the surface and evaporated Their hypothesis predicts that although the calcium abundance is low, magnesium and sulphur should be enhanced on Io's surface. The lines of these elements must be measured from outside the Earth's atmosphere. The implications of the detection of potassium for this hypothesis are not yet clear.

I point to an alternative interpretation of the preponderance of alkali metals in Io's cloud, which enables Io's surface to have a more typical composition Laboratory experiments by Cassidy and Hapke[11] show that sputtered sodium and potassium are hardly adsorbed on surfaces they impact. On the other hand, other elements are selectively adsorbed in direct proportion to their atomic weight. Cassidy and Hapke suggest that Io's surface microstructure may be rough enough for multiple scattering of the sputtered products to occur, thereby leading to a cloud rich in sodium and poor in other elements. My detection of potassium reinforces this suggestion. Sodium and potassium may occupy a larger portion of the cloud than of Io's surface material

A difficulty with this suggestion is that a significant portion of the sputtered products will be directed away from the surface so that they cannot directly suffer multiple scattering. This would result in inadequate enhancement of sodium relative to calcium. For selective removal of other elements to occur with high efficiency, it seems necessary for a process to exist which causes upward sputtered elements to reimpact the surface rather than to escape directly from Io's atmosphere. Such a process might be the effect of a plasma sheath[12] on ionised sputtered products, aided by particle scattering in Io's atmosphere.

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Angular momentum transfer to the inner Jovian satellites

THE transfer of angular momentum from a central rotating body in the presence of a magnetic field has been discussed in connection with the evolutionary history of the Solar System[1]. We here consider angular momentum transfer in the inner Jovian satellite system. Electron flux measurements near Io's flux tube[2], and theoretical estimates of the electric current flowing through Io's flux tube[3] are used to estimate the angular momentum transfer during the evolutionary history of the Jovian system. We find that, under certain conditions, those currents are sufficient to produce an angular momentum transfer from Jupiter equal to the present angular momentum of the inner satellites.

Consider the inner satellites to be those whose orbital radii are $\lesssim 15r_J$ (Jovian radii). Within this region the planetary magnetic field is orderly and adequately described as a magnetic dipole[4]. Unless the rotational angular velocity is constant throughout the system, potential differences will occur, resulting in current loops[1,3], as sketched in Fig. 1. The electric current flow across magnetic field lines results in a force which produces angular momentum transfer[1].

At an earlier stage of evolution, the material out of which a satellite formed is distributed around the parent body near the equatorial plane[1]. We assume here, for accuracy, that this material is spread out as a torus of small radius a and large radius r equal to the present orbital radius of the satellite. The angular momentum transfer ΔL from Jupiter to the torus in time Δt can be expressed as $\Delta L = rJBV\Delta t/c$, where J is the current density flowing through the torus, B is the magnetic field magnitude at r, $V(=2\pi^2ra^2)$ is the volume of the torus, and c is the velocity of light in a vacuum. The present angular momentum of the satellite is $L = 2\pi mr^2/T$, where m is the mass of the satellite, and T is its orbital period. For significant angular momentum transfer $\Delta L = L$, and

$$a = \left(\frac{mc}{\pi TJB\Delta t}\right)^{1/2}$$

Fig. 1 A sketch of an electric current loop in the Jovian magnetosphere. The arrows indicate the directions of current flow along and across dipole magnetic field lines.

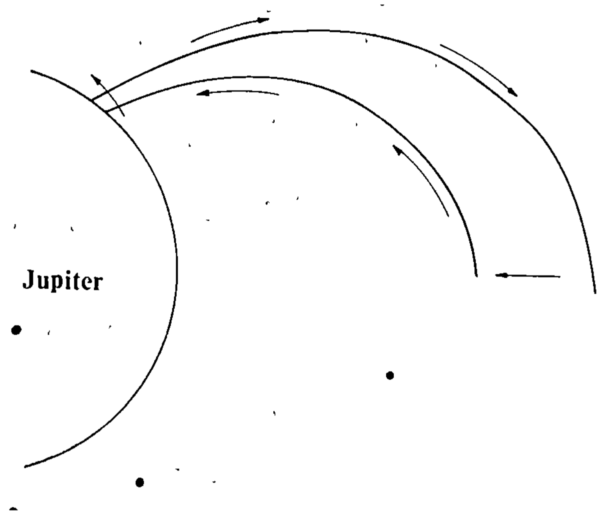

Table 1 Values of a, the small radius of satellite toruses

Satellite	Orbital radius (r_J)*	$a(r_J)$	a/r_S†
Amalthea	2.6	5.0×10^{-4}	0.3
Io	6.0	7.5×10^{-2}	3
Europa	9.5	8.8×10^{-2}	4
Ganymede	15.0	2.2×10^{-1}	5

*r_J, radius of Jupiter (present values[5]).
†r_S, satellite radius.

We now proceed to evaluate a for the inner satellites to find out if we arrive at plausible values for this radius. The masses and orbital periods of the satellites are well known[5]. The magnetic field in the magnetic equatorial plane is $B = 4(r_J/r)^3$ gauss (ref. 4). For Δt we use a rough estimate of the evolutionary time scale of the Jovian system, $\Delta t = 10^{16}$ s. The choice of a value for the current density J is based on the following considerations. Io has attracted attention due to the associated decametric radio bursts. It seems probable, however, that current systems are, or have been, present at other Jovian satellites. It has been reported[6] that Jovian radio bursts seem to be correlated with the phase of Europa, in addition to the well known correlation with Io's phase. Since there are no independent estimates of the magnitudes of the currents at satellites other than Io, we assume here that the current density J for all satellites is the same as that flowing at present along Io's flux tube. We adopt the working value of $J = 3\times10^{-7}$ A m^{-2} (ref. 3) which is compatible with recent measurements of electron fluxes near Io's flux tube[2]. (These electron measurements yield an upper limit of $\sim 2\times10^{-7}$ A m^{-2} for the current due to the electrons measured above the detector threshold of 460 keV.)

The resulting values for the small radius a of the evolutionary toruses are given in Table 1, where we observe that they are smaller than the orbital radii, and of the same magnitude as the present satellite radii. We conclude that the process we are considering is plausible.

This calculation suggests that angular momentum transfer via electric current loops could have been a significant process in the evolutionary history of the Jovian system. One of the critical parameters in the calculation is the magnitude of the electric currents. We have not assumed a value for this quantity, but instead we have used estimates of the current density in Io's flux tube which were arrived at independently to explain other physical phenomena, and which are compatible with recent measurements.

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Unusual series of type III-RS solar radio bursts

SOLAR radio emission on August 12, 1975, was unusual because of the large number of, and predominance of, type III-RS bursts ('reverse slope', positive frequency-drift type III bursts). These bursts seem to be second branches of U bursts and, as such, are consistent with only one current model. Four burst events have a Y-like profile and may be the first observations of the reflection of the burst exciter at a magnetic mirror.

Normal type III solar radio bursts drift rapidly toward lower frequencies. The negative drift ($df/dt < 0$) implies that an electron stream is moving upwards in the corona, exciting oscillation of the coronal plasma at progressively lower local electron plasma frequency and harmonics[1]. Variants of type III bursts include U bursts and type III-RS bursts. After drifting to a minimum frequency, U bursts turn and drift towards higher frequencies; the electron stream seems to reach a maximum altitude and be deflected back towards lower altitudes, possibly being guided along a magnetic arch[1]. Type III-RS bursts are rarely seen and seem to differ from type III bursts only in the sign of their drift rates; the electron stream thus seems to move downwards in the corona.

During the period 0457–1828 UT, August 12, 1975, the Durnten spectrograph (sweeping 125–1,000 MHz every 0.25 s) observed about 30 type III-RS bursts (Figs. 1*a* and *b*) and only ~ half that number of type III bursts. All bursts were observed in the approximate range 200–420 MHz, which is a window in local interference. Sensitivity decreases below 200 MHz. In spite of higher sensitivity in the range 400–750 MHz, few bursts extended to 500 MHz and none past about 600 MHz. The distributions of drift rate and duration are similar to those of type III bursts (Fig. 2). Two bursts were second branches of U bursts (Fig. 1*c* and *e*). Four events contain a type III-RS burst followed immediately by a type III burst, in a Y-like configuration (Fig. 1*d* and *e*). We will refer to these bursts as Y bursts. The Y bursts are isolated events and do not appear to be due to chance sequences of bursts of types III-RS and III; in the period 1453–1714 UT the only bursts recorded were three Y bursts.

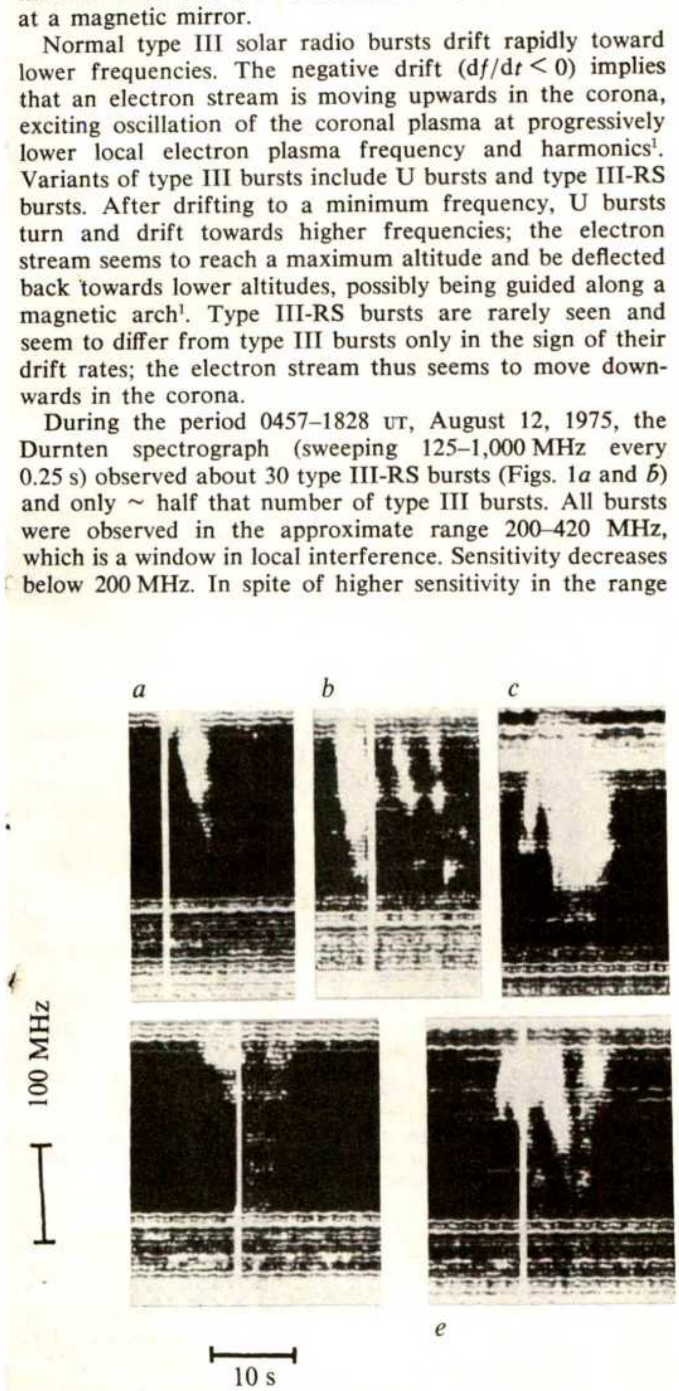

Fig. 1 Dynamic spectra of type III-RS bursts observed August 12, 1975. Frequency increases downwards, frequency ranges of examples are *a*, *b*, *d*, *e*, 200–500 MHz; *c*, 140–440 MHz. Times (UT) of examples are: *a*, 1714; *b*, 1102; *c*, 1102; *d*, 1651; *e*, 1507. The vertical lines are time markers; horizontal are transmitters.

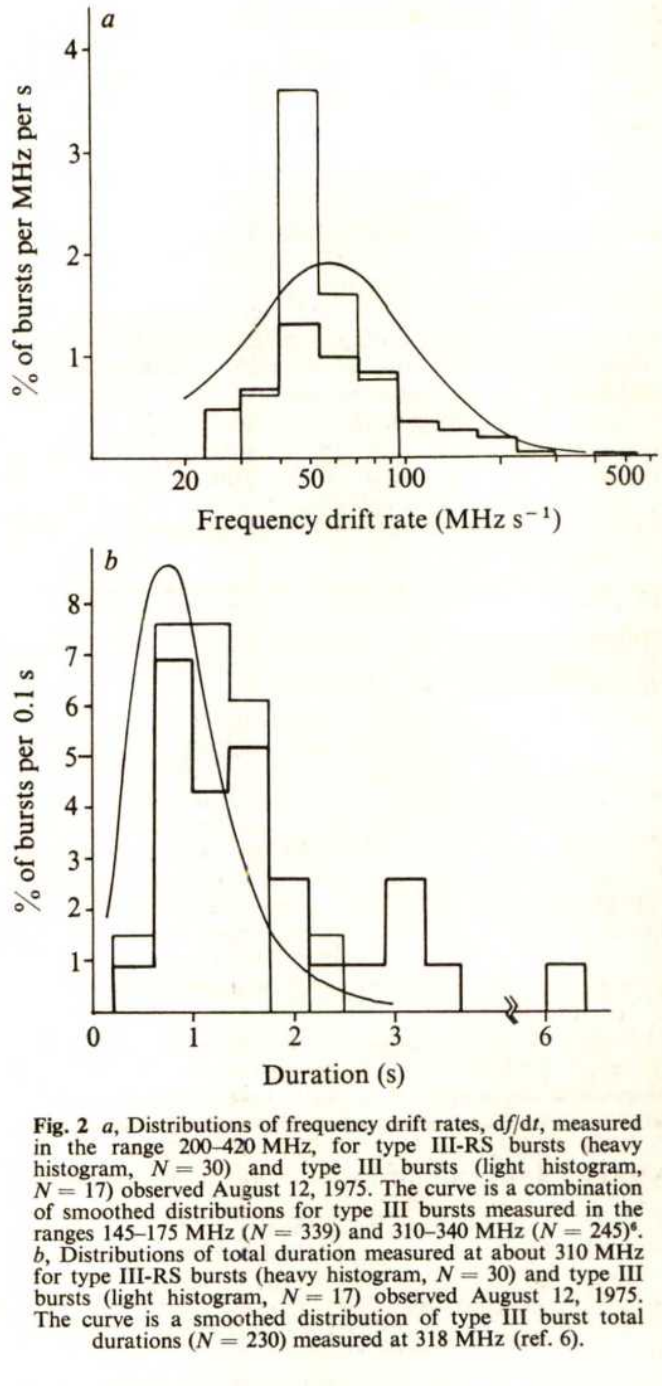

Fig. 2 *a*, Distributions of frequency drift rates, df/dt, measured in the range 200–420 MHz, for type III-RS bursts (heavy histogram, $N = 30$) and type III bursts (light histogram, $N = 17$) observed August 12, 1975. The curve is a combination of smoothed distributions for type III bursts measured in the ranges 145–175 MHz ($N = 339$) and 310–340 MHz ($N = 245$)[6]. *b*, Distributions of total duration measured at about 310 MHz for type III-RS bursts (heavy histogram, $N = 30$) and type III bursts (light histogram, $N = 17$) observed August 12, 1975. The curve is a smoothed distribution of type III burst total durations ($N = 230$) measured at 318 MHz (ref. 6).

The Y bursts seem to result from the reflection of a downward travelling electron stream at a magnetic mirror. The mirror points of the individual stream electrons would be distributed over a range in magnetic field strength and, thus, height. The mirroring region must, however, be contained in the few times 10^4 km between the burst source and the photosphere; the 10^5 km s^{-1} electrons could reflect and return to the source region in a time of the order of 1 s. About 3 s elapse in Fig. 1*e* between the disappearance of the type III-RS burst and that of the type III burst at 300 MHz.

Since type III-RS bursts are rare, we may assume that the bursts in this extended series are homologous, originating in the same general source region and from similar acceleration events. Two of the bursts being second (and stronger) branches of U bursts suggests that all the type III-RS bursts are second branches of U bursts. As simple as this interpretation appears, it disagrees with most U burst observations and models, where the second branch is weaker. If we allow that the situation may be reversed, we must exclude explanations of the weak second branch which are not reversible. Models using a major disturbance to the stream[2], continuous loss of energy[3], and less efficient emission travelling along the density gradient[4] cannot be reversed. On the other hand, the suggestion (ref. 5, and see ref. 2) that the electron stream becomes less dense due to diverging field lines is reversible; the electron stream entering a region of converging field lines would become denser and a more efficient source of emission and would be likely to mirror.

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Optical detection of Fe^{3+} in lunar plagioclase

NATURAL plagioclase from terrestrial sources exhibits a cathodal luminescence spectrum which consists in general of three emission bands[1,2]. The emission band in the blue region of the spectrum is thought to be associated with lattice defects[2], but the other emission bands are caused by transition-metal impurities present in concentrations of the order of 100–1,000 p.p.m. In particular, the emission band centred at 560 nm has been ascribed to Mn^{2+} ions substituting in Ca^{2+} sites, and the emission band on the edge of the infrared has been shown to be caused by Fe^{3+} ions probably in tetrahedral Al^{3+} sites[3]. The relative intensities of these emission bands vary from sample to sample, but the Fe^{3+} emission is often dominant in natural terrestrial plagioclases. These conclusions, concerning the nature of the luminescence centres involved, were arrived at by studying the emission characteristics of selectively activated, high purity, synthetic plagioclases[3]. Optical measurements presented there show that, using excitation spectroscopy, the detection of Fe^{3+} is possible in the plagioclase fraction of lunar fines.

Luminescence spectra of lunar plagioclases measured at room temperature do not show an easily discernible Fe^{3+} band; the presence of such ions in lunar plagioclase is, therefore, generally much less evident than in terrestrial samples of the same mineral. (The same is true of plagioclases of other extraterrestrial origin, such as those from meteorites.) But measurements of emission and excitation spectra at temperatures down to 4 K have shown that Fe^{3+} is certainly present in lunar and meteoritic plagioclases. Though measurements have so far covered only a few samples, it is likely that these results are typical; Fe^{3+} has been detected in all of the samples investigated. These results are consistent with the reported magnetic detection (electron spin resonance) of Fe^{3+} in a lunar anorthosite (15415)[4] and in the plagioclase fraction of rock sample 14053.47 and other lunar rocks[5].

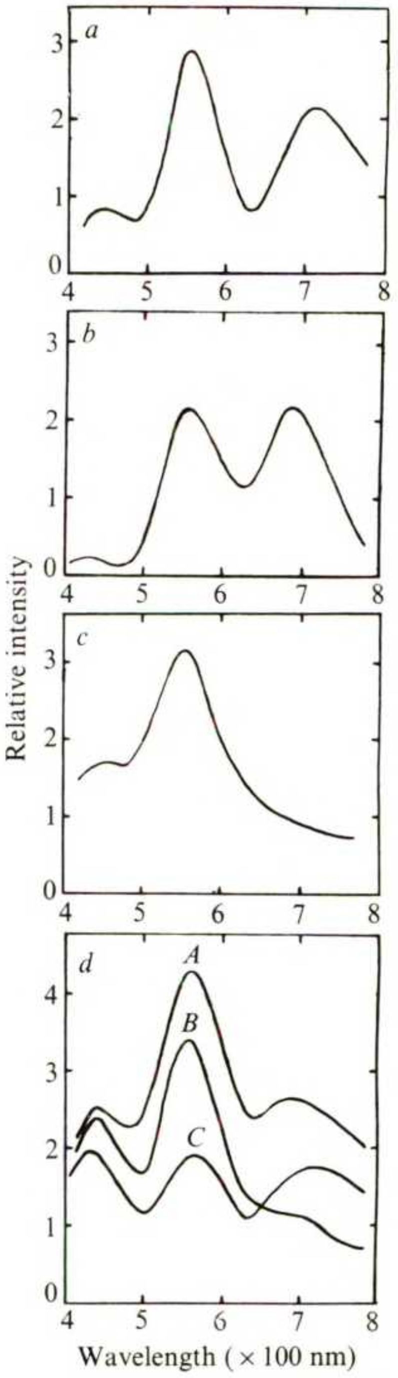
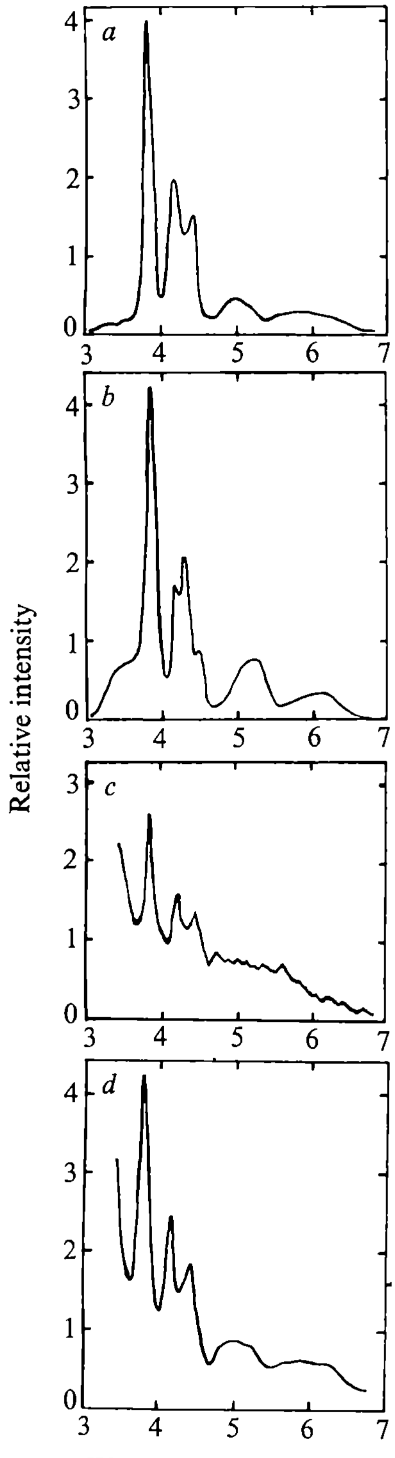


Fig. 1 Luminescence emission spectra under 10 keV electron excitation. *a*, Natural terrestrial bytownite (Emmons Suite No. 25), (see ref. 9); *b*, synthetic anorthite with 600 p.p.m. Mn^{2+} and 4,800 p.p.m. Fe^{3+} added; *c*, unseparated lunar fines 15061.611; *d*, lunar breccia 67455.3.1 (see text for explanation): *A*, 5 K, 8 Hz; *B*, 295 K, 8 Hz; *C*, 5 K, 29 Hz. The relative intensity scales (ordinates) for the different samples are unrelated. All spectra corrected for instrument response and measured at room temperature except where otherwise indicated.

Figure 1 shows the luminescence emission spectra of typical terrestrial, synthetic and lunar plagioclases. There is no detectable Fe^{3+} emission band in the spectrum of lunar fines 15061 in Fig. 1*c*. In Fig. 1*d* curve *B* is the emission spectrum of the lunar breccia 67455 at room temperature, in which the Fe^{3+} emission band is barely detectable. On cooling to 5 K this band becomes much more pronounced because the intensity of the Fe^{3+} emission has increased much more than the intensity of the green Mn^{2+} emission Curve *C* is a time-resolved spectrum which uses the fact that the Mn^{2+} emission has a longer lifetime than the Fe^{3+} emission to separate the bands still further. This was achieved by increasing the modulation frequency of the electron beam used for excitation from 8 to 29 Hz.

Fig. 2 Luminescence excitation spectra of the Fe^{3+} emission *a*, Natural terrestrial plagioclase (Emmons Suite No. 25, 77% anorthite)[9]; *b*, synthetic anorthite with 4,800 p.p.m. Fe^{3+} added; *c*, lunar fines 15601.611; *d*, lunar breccia 67455.3.1. Relative intensity scales (ordinates) for different samples are unrelated. A Wratten 70 filter was used to isolate the Fe^{3+} emission. All spectra measured at 5 K



The spectral position of the Fe^{3+} emission peak varies somewhat from sample to sample, and some correlation with plagioclase composition has been found[3].

Though Fe^{3+} can be detected in emission, particularly at low temperatures, the most sensitive and conclusive method of detection is that of luminescence excitation spectroscopy. Using this method the total emission in the deep red or near infrared is isolated by a filter and monitored using a photomultiplier as the sample is excited by intense monochromatic light scanned in wavelength. Figure 2 shows the Fe^{3+} excitation spectra for the same samples for which the emission spectra were presented in Fig. 1. The Fe^{3+} ligand-field bands can be recognised easily in the excitation spectrum of the fines 15601 in Fig. 2*c* whereas the emission spectrum in Fig. 1*c* did not show any evidence of the presence of Fe^{3+} Considering the similarities of the spectra in Fig. 2, there can be little doubt that lunar plagioclases also contain Fe^{3+}, although in lower concentrations than in natural terrestrial samples. These spectra are similar to those of Fe^{3+} in $LiAl_5O_8$ obtained by Pott and McNicol[6] and also similar to the absorption spectrum of Fe^{3+} in an iron orthoclase determined by Faye[7].

Excitation spectra of the Mn^{2+} emission in such plagioclases have also been obtained, thereby providing additional evidence as to the nature of that centre, but these will be presented elsewhere.

Luminescence excitation spectroscopy is obviously a sensitive and effective way of obtaining absorption spectra of luminescent species from powdered or mixed crystalline samples[8]. The method does not require any mineral separation at all, neither does it require highly polished surfaces which are necessary for electron microprobe work.

Experimental details and assignments of the observed ligand-field bands of Fe^{3+} and Mn^{2+} in plagioclase feldspars, including calculations of ligand-field parameters, will be given elsewhere.

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Stratospheric measurements of H_2O and the diurnal change of NO and NO_2

We have flown a radiometer from a balloon (a detailed description of which will be published elsewhere), which senses infrared emission from minor stratospheric constituents on the atmospheric limb. To provide a vertical profile of their concentration, the input mirror of the radiometer scans between 7° below and 2° above the horizontal, as illustrated in Fig. 1. The vertical field of view is 0.4°. Every 5.5 min the same mirror scans to an internal black body and to a space view at 18° upwards, thereby providing a two-point calibration.

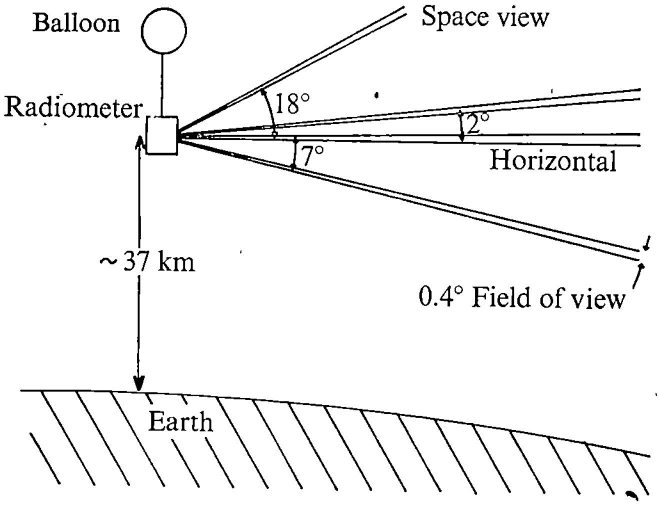


Fig. 1 Viewing geometry.

The optical system of the radiometer contains a rotating chopper which modulates incoming radiation at 195 Hz, and beam-splitters which divide the radiation between 3 channels with superimposed fields of view. Each channel contains an interference filter which selects the emission band of interest, a cell of the gas to be measured in which the gas pressure is modulated at a frequency near 20 Hz, and an infrared detector. The pressure modulator cell[1,2] acts as a filter of very high resolution (down to 3×10^{-3} cm^{-1}) which selects emission from the gas to be measured. The resulting increased sensitivity to this gas greatly reduces the relative sensitivity of the radiometer to changes in thermal emission from its input optics, and to emission from unwanted gases whose bands overlap the spectral region being observed. The detector receives components of radiation at both 195 Hz and 20 Hz, and by comparing the signals at each frequency we can almost completely compensate for the emission from unwanted gases. The device can be used even for corrosive gases, and has worked well, for instance, for NO.

The three channels measured emission from NO near 1,900 cm^{-1}, NO_2 near 1,600 cm^{-1}, and water vapour from 500 to 200 cm^{-1}. The 195 Hz water signal also measured the attitude of the radiometer by sensing the tropopause, above which the emissivity of the atmosphere drops from 1 to 0.7 within 3 km.

The sensitivity of the radiometer to each gas was determined in the laboratory by measuring the absorption of the gas in a cell in series which each pressure modulator cell. This sensitivity calibration is then independent of spectroscopic data, although such data were used to modify the water vapour calibration to compensate for different source and absorber temperatures between the laboratory and atmospheric measurements.

We present some results from two flights from the CNES launch site at Gap (44.2°N, 6°E) in southern France. The radiometer remained at its float altitude near 37 km for 5 and 7 h respectively, drifting almost due west towards 44°N, 0°E on each flight.

Figure 2 shows our measurements of the water-vapour mixing ratio. Between 15 and 30 km we find similar magnitudes to those of other workers[3,4], but above 25 km we find a substantial increase—reaching about a factor 2 by 35 km. Our systematic errors (due to uncertainties in altitude and attitude of the radiometer and in atmospheric temperature) give rise to significant absolute errors, but to rather smaller errors at one height relative to another. The increase in mixing ratio between 25 and 35 km is therefore at least a factor of 1.9—the results cannot be interpreted as a constant water mixing ratio. This increase supports the hypothesis that methane oxidation takes place in this region[5], where the methane concentration decreases rapidly with increasing height[6,7].

Few measurements of water vapour above 30 km have been reported. Our results are different from those of de Jonckheere[8], but are consistent with measurements by Scholtz *et al.*[9] at 45 km. At higher latitude, Evans[10] observed the mixing ratio to approximately double between 32 and 42 km, with a pronounced peak at 45 km.

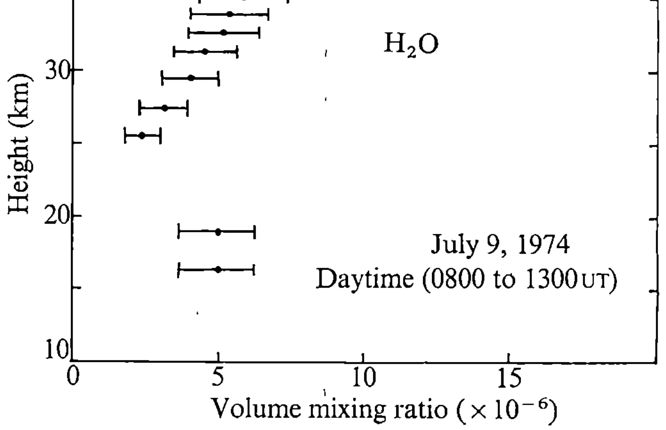
The later flight gave the first simultaneous measurements of changes in stratospheric NO and NO_2 from night to day. Our preliminary results are displayed in Fig. 3, and we find rather larger concentrations of these gases than other workers in the field. The probable errors at the present stage of analysis are less than a factor of 2, and again are mostly systematic errors in absolute concentrations. Changes in relative concentrations with altitude and time are much less uncertain.

The night-time concentration of NO was barely detectable relative to instrument noise, and was probably less than 0.8×10^{-9} by volume. The time of change and its sense (it rose to 15×10^{-9} by volume) agree with Patel's measurement at the single height of 28 km[11], although our daytime value is ~ 1.5 times larger, and over 5 times larger than daytime measurements by Schiff *et al.* up to 30.6 km (ref. 12).

Our NO_2 values are also somewhat larger than those obtained by solar absorption spectroscopy[13,14]: they drop from 35 to 16×10^{-9} by volume at a height of 36 km. They are lower and have the opposite diurnal variation to those of Brewer[15], but they are in broad agreement with those of Harries (personal communication).

Since the atmospheric path emitting to the radiometer is at least 300 km long, and the radiometer looks in a random direction relative to the Sun, the rapid rates of change of NO and NO_2 predicted at dawn by Whitten and Turco[16] would be observed as time constants of ~ 15 min. We observed such a

Fig. 2 Water-vapour mixing ratio. The values < 20 km are from the 195-Hz signal, those > 25 km from the 20-Hz PMR signal. Random errors are equivalent to $\pm0.1\times10^{-6}$ at 25 km and $\pm0.4\times10^{-6}$ at 35 km. The data cannot be interpreted as a uniform mixing ratio.



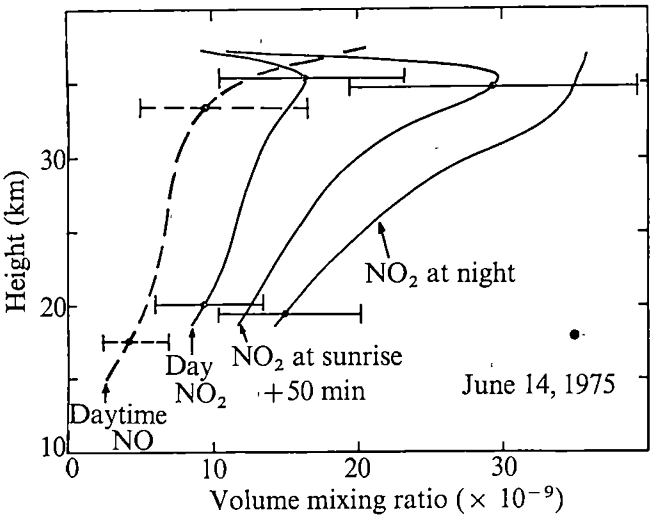


Fig. 3 NO and NO_2 mixing ratios. The errors are approximate, and contain both systematic and random components. Random errors are less than 0.5×10^{-9} for NO_2, and about 1.5×10^{-9} for NO. Systematic errors will be reduced by further analysis.

time constant for NO_2 at 36 km, but below 30 km the initial fall just after dawn was followed by a further slow fall over the next 2 h. Similarly, the lower altitude NO concentration continued rising for some hours after dawn. Within the errors of the analysis we have so far achieved, the sum of NO and NO_2 concentrations remains constant from night to day.

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Possible climatic impact of tropical deforestation

OF the various mechanisms suggested by which man might change the planetary climate, the removal of tropical rain forests to increase arable acreage seems to be one of the more imminent. For this reason we selected this as one of the first problems to be tested in our recently updated climate model. Bearing in mind the fallibility of computer simulations, we find overall global cooling and a reduction in precipitation: a larger tropical reduction being almost balanced by a subtropical increase.

The revised two-dimensional (zonal) atmospheric model (ZAM2)[1] uses basic conservation equations and parametrises the meridional transport by horizontal eddies using both model dependent and prescribed diffusion coefficients[2,3]. Vertically, the atmosphere is divided into nine pressure levels, with three above the tropical tropopause. At each latitude of the 10° latitude grid, the lower boundary is considered to be subdivided into ocean, sea-ice, and land of differing types and elevations to properly represent the planetary surface. Although this does not give ZAM2 the spatial coherence of continents and oceans it does permit realistic treatment of boundary exchanges and energy and water balance. As the conditions at each latitude band change, the surface responds appropriately. Soil moisture is simulated by maintaining a surface water depth representing the running sum of precipitation, evaporation and runoff (a variable exponential loss rate designed to account for both runoff and percolation).

Roughly 34% of the equatorial zone from 5°N to 5°S is covered by tropical rain forest. To investigate the climatic effect of its removal, three simulation runs were made with ZAM2: first, a control run designed to represent present conditions with the rain forest albedo set at 0.07; second, the complete removal of the tropical rain forest simulated by increasing the albedo to 0.25, combined with an increased runoff rate and decreased evaporation rate; and third, 'wet' deforestation simulated by increasing the albedo to 0.25 only. Except for these modifications, all three simulations started with the same initial conditions and were run to equilibrium (which was determined by monitoring the rate of change of selected parameters, and required approximately six months integration). The constant sun version of ZAM2 was used, in which diurnal and seasonal variations are suppressed by imposing the annual mean solar flux appropriate to each latitude. This does not allow the strong north–south temperature gradients typical of winter, but has been shown to reproduce annual mean atmospheric conditions quite well[4].

The chain of consequences following removal of the tropical rain forest as deduced from the ZAM2 results can be summarised as follows: deforestation → increased surface albedo → reduced surface absorption of solar energy → surface cooling → reduced evaporation and sensible heat flux from the surface → reduced convective activity and rainfall → reduced release of latent heat, weakened Hadley circulation, and cooling in the middle and upper tropical troposphere → increased tropical lapse rates → increased precipitation in the latitude bands 5 to 25°N and 5 to 25°S and a decrease in the Equator–pole temperature gradient → reduced meridional transport of heat and moisture out of equatorial regions → global cooling and a decrease in precipitation between 45 and 85°N and at 40 and 60°S. The changes were of essentially the same character in both perturbation cases, and seemed to be somewhat larger for the case of increased surface albedo only. We believe this is due to the greater cloudiness which occurs with the 'wet' deforestation. Contrary to expectation, most of the increase in cloudiness on this run occurred between 5 and 45°N.

Fig. 1 Predicted temperature changes due to removal of the tropical rain forest.

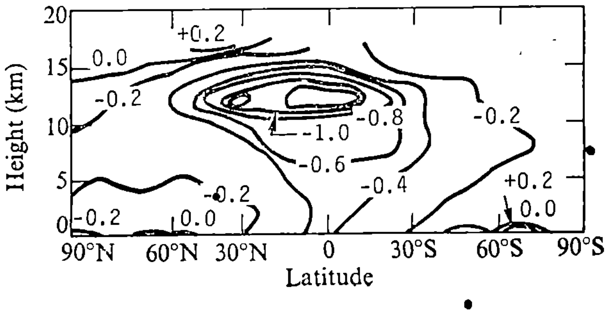


Table 1 Mean global water balance

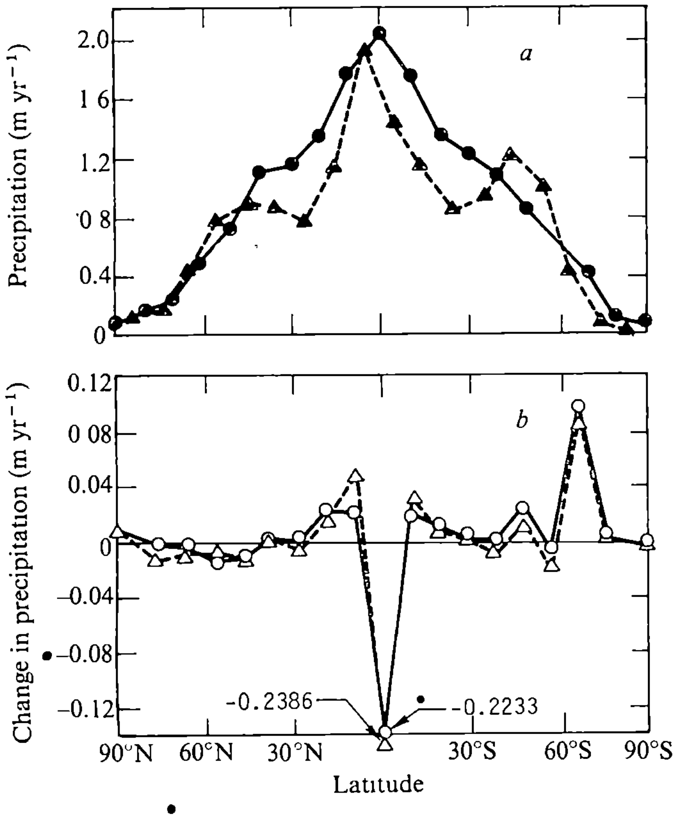
	Control	Complete deforestation	Albedo change only
Atmospheric water vapour content (gm cm^{-2})	2.4731	2.4032	2.394
Evaporation (gm cm^{-2} d^{-1})	0.33323	0.32983	0.33016
Rainfall (gm cm^{-2} d^{-1})	0.33305	0.32996	0.33169
Cloudiness	0.493	0.490	0.491
Atmospheric residence time (d)	7.4236	7.2847	7.2499

Figure 1 shows the latitude and height distribution of tropospheric temperature change for the complete deforestation simulation (perturbed minus control). The maximum temperature reduction below the tropical tropopause demonstrates the influence of reduced convective activity and subsequent reduced latent heat release. The poleward spread of this effect contributes to the decrease in static stability and increase in precipitation at 5 to 25°N and 5 to 25°S. Globally, the average surface temperature decreased 0.20 K for the completely deforested simulation and 0.30 K for the case of increased surface albedo only. The larger decrease in temperature in the latter case emphasises the importance of surface albedo and demonstrates the often overlooked tendency for self-compensation of geophysical processes in the real world. This represents the first time we have found a global temperature change not accompanied by greater surface changes at the poles than at the equator.

The zonal mean precipitation computed by ZAM2 during the control run is compared with observations in the upper part of Fig. 2. The lower part of the figure shows the changes in precipitation resulting from the two simulations of deforestation. The precipitation decrease in high northern latitudes reached 1.5 to 2.6% but amounted to only 0.9 and 0.7% when averaged over the globe for the two cases. The increases at 70°S are probably due to increased evaporation resulting from increased katabatic flow off Antarctica, but the reason for this increased flow has not yet been determined.

Table 1 summarises the global water budget as computed by the three model runs. These show what has now come to be a familiar pattern; global cooling leads to reduced atmospheric water vapour content (reinforcing the cooling by a reduced 'greenhouse' effect). This in turn leads to a reduction in global mean precipitation, which is consistently smaller than the reduction in precipitable water because of an acceleration of the atmospheric water cycle (revealed by the decrease in residence time of water vapour in the atmosphere). These effects are in turn accompanied by an even greater cooling in the upper tropical troposphere, a steepening of lower tropospheric lapse rates, a lowering of the tropical tropopause and a warming of the lower tropical stratosphere (see Fig. 1).

Fig. 2 Precipitation predicted by ZAM2 *a*, Comparison of control case (●) with observations compiled by Sellers[5] (▲) *b*, Change in precipitation (perturbed minus control) for complete deforestation (○) and albedo change only (△).



In this type of experiment the results should be viewed primarily as indications of possible change and not as absolute indicators. Although as yet, no claim can be made that this cascade of events would occur in the real atmosphere, it is consistent with the prevailing conception that lapse rates in the lower troposphere and the height of the tropical tropopause are determined largely by moist convection. Also, in a parallel study, the model duplicated quite well the published results[6] of a three-dimensional general circulation model test of the climatic impact of increased albedo by denudation of the Sahara.

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Evidence for a Proterozoic greenstone belt from Snow Lake, Manitoba

THE Archaean-Proterozoic boundary, it has been suggested, marks a fundamental change in the behaviour of the Earth, ending an early phase of cratonisation and the beginning of a period characterised by stable shelf sedimentation and linear orogens[1,2,3]. Almost all Archaean terrains, other than those dominated by granulites and migmatites, seem to be characterised by a variety of granitoid rocks intimately associated with ultramafic through mafic to calc-alkalic sequences, and their sedimentary associations These greenstone belt assemblages are remarkably similar from one craton to another; they are usually folded into keel-shaped troughs, subjected to greenchist facies metamorphism and usually show similar structural styles which suggest that they were acted on by predominantly vertical forces Their

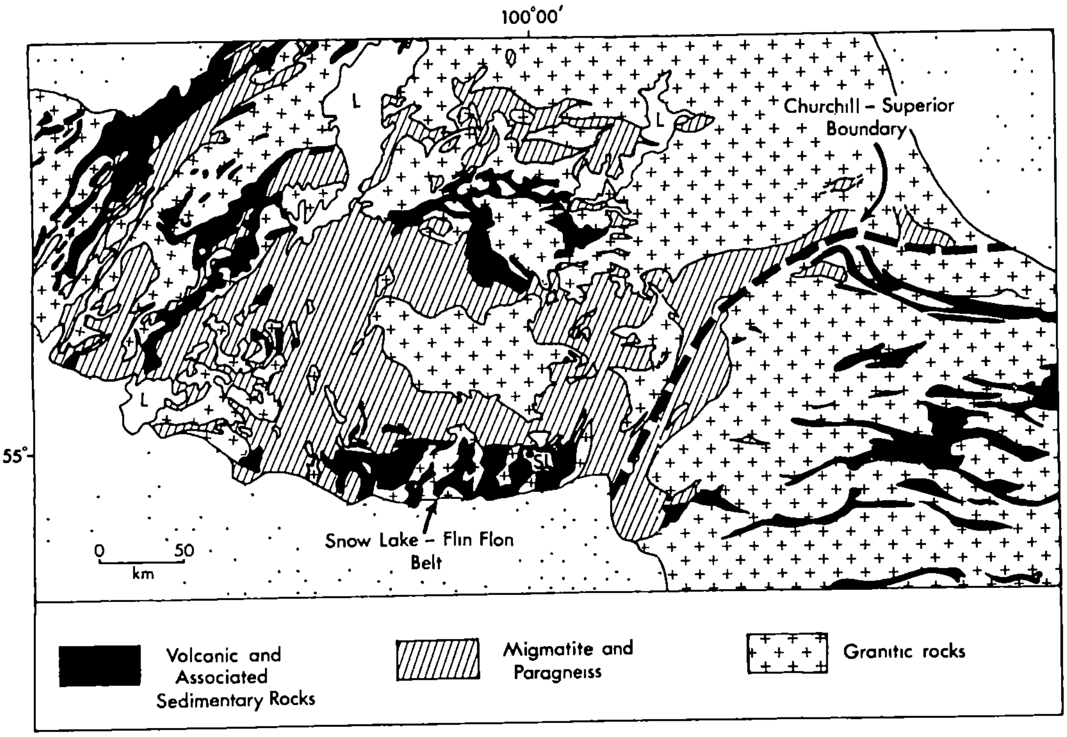
100°00'
55°
Churchill - Superior Boundary
L
S.L.
0 50 km
Snow Lake - Flin Flon Belt
Volcanic and Associated Sedimentary Rocks
Migmatite and Paragneiss
Granitic rocks

Fig. 1 Regional setting of the Flin Flon–Snow Lake belt at the southern margin of the Churchill Province. The Superior Province lies to the east. L, lakes; S.L., Snow Lake.

alleged antiquity (2,000 Myr or more) and widespread distribution have been attributed to either a change in the geothermal gradient as the Earth became colder[3] or the existence of a relatively thin, Archaean, continental crust[4,5].

Detailed proposals for the origin of greenstone belts are more varied and include models that compare the volcanic–sedimentary sequences with : (1) primitive crustal remnants[6], (2) volcanism associated with continental rifting[1] and (3) island arc sequences[7]. The last of these is particularly interesting because it implies that plate tectonic processes were common in the Archaean and, as a corollary, that greenstone belts or their equivalents were formed throughout geological time. This note presents some new Rb–Sr data which indicate that not all greenstone belts are restricted to the Archaean. If this conclusion is accepted then the uniqueness of Archaean magmatic processes diminishes, and we are faced with attributing the origin of greenstone belts to mechanisms similar to those we find operating at the present time.

The Flin Flon–Snow Lake volcanic–sedimentary belt at the southern margin of the Churchill Province in northern central Manitoba and Saskatchewan (Fig. 1)[8,9] has traditionally been regarded as Archaean on the basis of its approximate east–west trend and its similarity to Archaean greenstone belts in the nearby Superior Province to the east[10]. Numerous high grade base-metal deposits related to the volcanic successions make this one of Canada's major metal-producing areas. Deformation, metamorphism and plutonism have been ascribed, in large part, to the Hudsonian Orogeny[11]. Age determinations have indicated some evidence for an Archaean history for the Hanson Lake area, near the western part of the belt[12], but of an Aphebian age (1,700–1,800 Myr) of magmatism for the Flin Flon–Snow Lake area (refs 13, 14 and G. R. Josse, unpublished).

Although not continuous with the type succession, the major rock units at Snow Lake are correlative with the Amisk metavolcanics and overlying Missi metasediments at Flin Flon[15,16]. Full details of the Amisk and Missi Groups at Snow Lake are given in Table 1. The entire succession has been deformed into three major fold sets and metamorphosed with grade increasing from the biotite zone in the south to the sillimanite–almandine zone in the gneiss dome terrain to the north (see Fig. 2). Major stratigraphical units can be traced across the isograds. The cores of the gneiss domes are granitoid rocks with chemical compositions similar to the more felsic of the volcanics.

Table 2 summarises the whole-rock Rb–Sr isochron results from six suites collected from the Snow Lake area. The localities are shown in Fig. 2. Of the six isochron ages listed in Table 2, none is Archaean, none is older than

Table 1 Stratigraphy of Snow Lake

Missi Group	Arkosic siltstone and sandstone, typically cross-bedded; minor conglomerate, greywacke	Post-Missi (?) intrusions: gabbro, diorite, granodiorite, granite
	———Minor Unconformity———	
Amisk Group	Pelite and greywacke turbidites	Plutonic and hypabyssal equivalents (?): 'Quartz-eye' dacite Granitoid gneiss domes of Herblet and Squall Lakes
	Interlayered tholeiitic and calc-alkalic volcanics; basalt, andesite, and dacite breccias, tuffs and flows; calcareous and pyritic tuffs; related intrusions	
	Pillowed and massive basalt flows; related intrusions	

Table 2 Analytical results

Locality		Petrographic notes	No. of points	Initial ratio	Age (Myr)	MSWD
A	Amisk Group—due south of Snow Lake	Amphibolite, biotite-hornblende gneiss, garnet-biotite gneiss and schist derived from pillowed tholeiite, basalt breccia, andesite, and fine-grained andesite and dacite pyroclastics	5	0.7020±0.0005	1,720±45	2.3
B	Amisk Group—due north of Snow Lake	As above	4	0.7017±0.0007	1,720±40	3.4
C	Triangle Dome	Very uniform pink, granoblastic, medium-grained biotite-hornblende granites with approximately equal amounts of microcline, sodic plagioclase and quartz	4	0.7017±0.0010	1,750±70	2.8
D	East Dome	As above	6	0.7024±0.0018	1,720±110	5.3
E	Squall Dome	Recrystallised uniform leucogranodiorites, one with minor garnet; schistose and gneissic variants with up to 10 percentage biotite plus muscovite, also lens of hornblende-biotite metadiorite	4	0.7014±0.0009	1,690±60	3.1
F	'Quartz-Eye' Dacite	Biotite-quartz-plagioclase schist, with variable small amounts of garnet and hornblende. Polycrystalline quartz 'eyes' derived from phenocrysts in fine granular matrix. Contains inclusions of enclosing metavolcanics	5	0.7019±0.0006	1,510±110	3.6

All errors given at the 2σ level. All ages calculated using $\lambda = 1.47 \times 10^{-11}$ yr^{-1}.

1,750 Myr and all reflect a Proterozoic event which we believe corresponds to the age of magmatic activity. Other than that of the 'quartz-eye' dacite, the isochron ages fall within a fairly restricted range of between 1,750 and 1,690 Myr. We have also listed in Table 1 a statistical parameter, the mean square of the weighted deviates (MSWDs)[17], which gives some idea of how well the points fit a straight line. For our isochrons the MSWDs indicate that the points fit the line within experimental error.

It is possible to interpret the analytical data in two different ways, one of which we have mentioned. The other interpretation assumes that the rocks are considerably older and that the Proterozoic ages are the result of a resetting and updating of the Rb–Sr systems by the Hudsonian Orogeny. This we feel unlikely on the following grounds: First there is very little scatter of the data other than what can be attributed to analytical uncertainty. The low MSWDs (between 2 and 5) suggest that there is little 'geological noise' of the type to be expected from a disturbance of the Rb–Sr systems. Second, all of the rocks have relatively low, uniform initial $^{87}Sr/^{86}Sr$ ratios which suggest that these rocks have not had an extensive earlier history. Third, single-stage model-lead ages for the stratabound sulphide deposits, which on geological grounds are coeval with their host rocks, predominantly volcanics of the Amisk Group, fall between 1,900 and 1,780 Myr (ref. 14). Although

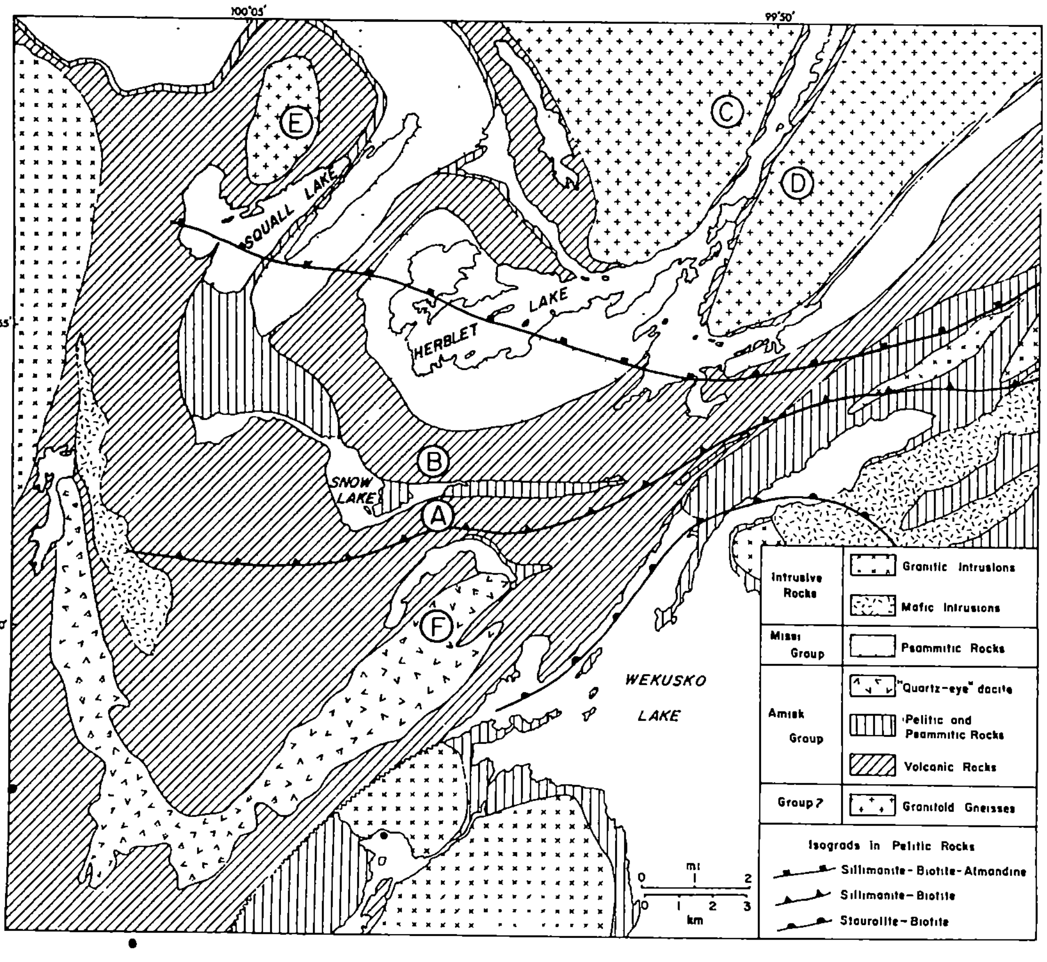

Fig. 2 Detailed map after Froese and Moore[16] showing sample localities A–F. For locality descriptions see Table 2.

these ages are strongly model-dependent the isotopic data can be interpreted to be consistent with our Rb–Sr results. Finally, if the Hudsonian Orogeny were responsible for the complete resetting of the Rb–Sr systems then a process would have to be invoked which behaved independently of chemical composition, grain size and metamorphic grade.

We prefer the simplest interpretation of the isotopic data, which considers the volcanics and gneiss domes as magmatic equivalents erupted just before the onset of the Hudsonian Orogeny. It is significant that there is no evidence either in our data or those of other laboratories for an Archaean crustal history. In spite of the similarities of the rocks at Snow Lake to Archaean greenstone belts, the radiometric data consistently indicate Aphebian volcanism. The close association of stratabound sulphide deposits to Proterozoic, rather than Archaean, volcanicity has some profound implications, particularly in the context of base-metal exploration in the Canadian Shield.

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Evidence for pre-Jurassic subduction in western Antarctica

THE South Shetland Islands (from King George Island to Livingston Island) are situated on a small crustal plate bounded by incipient back-arc spreading along the axis of Bransfield Strait to the east, a well defined oceanic trench to the west (along which subduction has apparently now ceased) and transverse faulting to the north and south[1] (Fig. 1). The discovery of glaucophane-schists on Smith Island (Fig. 1, inset) suggests that subduction may have begun as early as the Upper Palaeozoic.

The geological history of the South Shetland Islands (which includes the Elephant and Clarence Islands group) is dominated by continuing volcanism from the (?) Jurassic to the present[2]. A structurally deformed sedimentary sequence of presumed Upper Palaeozoic age crops out on Livingston Island[3,4]. Structurally deformed phyllites, schists and rare marbles, constituting the Elephant and Clarence Islands group, have been assigned a (?) Precambrian age[4,5] but Smith Island is known only to consist of "basement rocks" (see ref. 6).

Cape Smith on Smith Island, and Barlow Island are formed of grey-green strongly schistose rocks exhibiting fine colour banding (mainly shades of green but including dark, grey-blue horizons) and small-scale structural features; the banding is dominantly parallel to the schistosity but marked local discordances occur. A buff coloured, fine grained, clastic dyke, indurated and pinched out laterally, cuts the schistosity at Cape Smith, and quartz veining, both concordant and discordant, also occurs.

Thin-section analysis of specimens collected from the localities has revealed five metamorphic rock types: epidote-quartz-garnet-glaucophane-semischist; epidote-albite-chlorite-glaucophane-schist; epidote-chlorite-glaucophane-schist; epidote-glaucophane-schist; and an epidote-albite-chlorite rock.

The mineralogy of these rocks is relatively simple. Yellow, slightly pleochroic epidote (pistacite) forms layers of tiny granules and stubby prisms, and idiomorphic acicular glaucophane (α=colourless; β=deep blue; γ= colourless) is aligned parallel to the schistosity. Mosaic quartz and albite are the commonest associates and probable metamorphic differentiation of these four phases has created layers and lenses virtually exclusive of other minerals. Prochlorite (?) is also present and minute granules of accessory sphene (?) form discontinuous, sinuous trains. Xenomorphic pale yellow garnet (composition undetermined) occurs in one specimen, often altered to a red-brown mineral and intimately associated with epidote, both externally and internally. This specimen also contains radiating sheaves of stilpnomelane largely restricted to the epidote-rich layers intercalated between laminations of finely crystalline quartz (possibly originally chert). With that exception, all of the rocks have a basic composition[7] and probably represent original basic igneous rocks.

The most conspicuous structural feature is a regular schistosity, the planes of which exhibit a mineral lineation. Both of these features have subsequently been deformed, resulting in tight asymmetric folds of several centimetres wavelength. Kink bands of 1 or 2 mm diameter are irregularly developed. Stereographic projection of limited structural data also indicates two phases of deformation.

Although these rocks are the first glaucophane-schists to be recorded *in situ* from western Antarctica, two glaucophane-schists were dredged from south of Clarence Island[8], and five from the northern Scotia Ridge by RRS Shackleton in 1972 (Fig. 1).

No inference can yet be made regarding the provenance of the northern Scotia Ridge specimens. The ubiquitous association of ultrabasic (mostly serpentinitic) rocks with glaucophane-schists may be represented here by the serpentinite-dunite of Gibbs Island and the glaucophane-schists of Smith Island, which suggests that the specimens described by Tyrrell originated within the South Shetland Islands. Texturally, Tyrrell's specimens are distinct from those of Smith Island.

Geophysical evidence suggests that a metamorphic basement stretches from the Elephant and Clarence Islands group as far south as Livingston Island[6]; this probably extends to include Smith Island.

Apart from textural differences, all of the glaucophane-bearing rocks from the South Shetland Islands have similar mineral assemblages which are atypical of both greenschist and glaucophane-schist facies. In particular, the coexistence of epidote, garnet and soda-amphibole is typical of conditions transitional between these two facies[9].

Lithological and structural similarities[10,11] between the Elephant and Clarence Islands group and the South Orkney Islands are supported by geophysical evidence[12]. The latter islands largely comprise metamorphic rocks

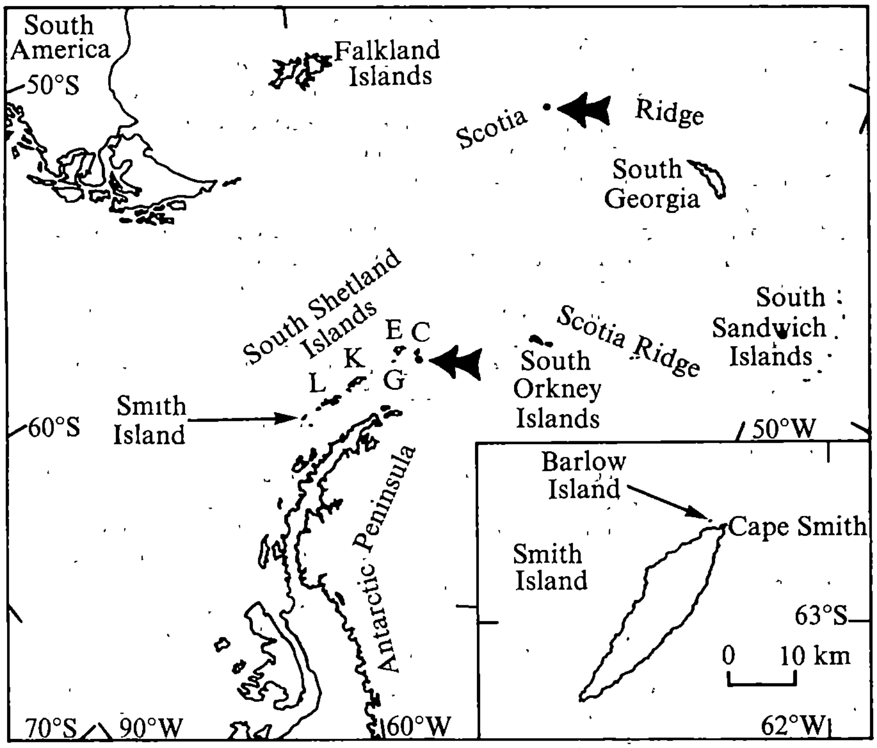

Fig. 1 Map of the Scotia Ridge showing the occurrences of glaucophane-bearing rocks. Arrowed solid circles, dredge sites; C, Clarence Island; E, Elephant Island; G, Gibbs Island; K, King George Island; L, Livingston Island. Inset, Smith Island.

belonging to the highest grade of the greenschist facies[7] but with a local development of quartz-albite-sillimanite-schists at Mansfield Point on Coronation Island (South Orkney Islands) which may possibly be attributable to metasomatism from an unknown source at depth.

Whereas lithological differences between the deformed metamorphic rocks of the Antarctic Peninsula and those of the offlying islands preclude correlation[4,13], the contrasting physical conditions of metamorphism have not been emphasised previously. The rocks of the Antarctic Peninsula, metamorphosed under high-temperature, low-pressure conditions, have been specifically compared with the Ryoke–Abukuma Metamorphic Belt of Japan[14]. Conversely, those of the South Shetland and South Orkney Islands, formed in low-temperature, high-pressure conditions, are more similar to those of the Sanbagawa Metamorphic Belt of Japan[15,16]. It is therefore proposed that a paired metamorphic belt, analogous to the Ryoke–Abukuma/Sanbagawa paired belt, exists in western Antarctica, and its formation is ascribed to the subduction of oceanic crust along a Benioff Zone beneath either an island arc or a continental margin[17]. The relative positions of the inner and outer metamorphic belts indicate subduction from west to east.

There is no evidence to indicate conclusively the age of the initial metamorphism of the rocks of the metamorphic complex. A pre-Jurassic age is, however, indicated by the unmetamorphosed and largely undeformed Jurassic igneous intrusions of the Antarctic Peninsula[13]. Radiometric ages[18] suggest that a significant metamorphic and deformational event occurred in the Upper Palaeozoic but this may be considered a minimum age. Although there is no radiometric evidence to suggest a metamorphism before the Upper Palaeozoic, it cannot be shown that the formation of the two metamorphic belts actually occurred at this time, but they must have existed to account for the similar radiometric ages from the Antarctic Peninsula and the South Orkney Islands. Accepting the reconstruction that there was a continuous Andean–western Antarctic cordillera at least until the late Mesozoic[13], it is interesting that a crossite-bearing, paired metamorphic belt present in central Chile has been assigned[19] an Upper Palaeozoic age.

The major fault[17] or crustal suture[20] which seems to characterise paired metamorphic belts can only be inferred at present, but it may be expected on the South Orkney Islands platform (perhaps located along the 2.5 km deep, buried trench to the south of the islands[21]) and in Bransfield Strait, where such a feature may have had a major influence on the evolution of the back-arc spreading.

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Ceramic steel?

ZIRCONIUM DIOXIDE (zirconia) has three allotropes: monoclinic, tetragonal and cubic. The transition between the first two involves a large volume expansion which prevents the refractory properties of pure zirconia being used in structural ceramics. The disruptive phase transformation can be suppressed by total stabilisation in the cubic form, but it is generally recognised that the most useful mechanical properties are obtained in multiphase material known as partially-stabilised zirconia (PSZ). Garvie and Nicholson[1] have demonstrated that a fine-scale precipitate of monoclinic zirconia in a cubic stabilised matrix enhances the strength of PSZ. Here we report that a dispersion of metastable tetragonal zirconia in cubic zirconia can also be achieved, and that this gives rise to another, more powerful, strengthening mechanism.

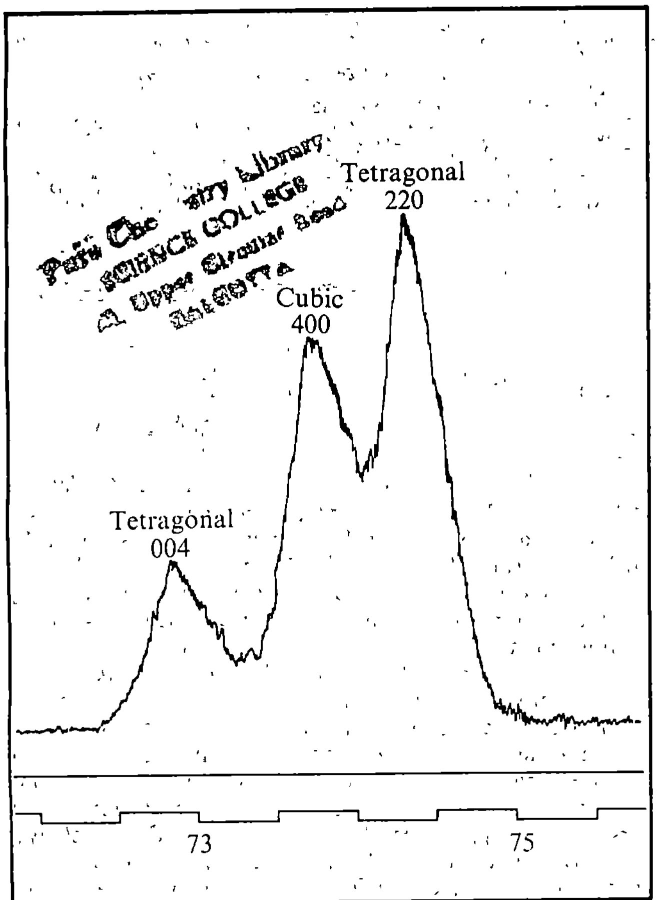

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Fig. 1 X-ray diffractometer trace of the {400} peaks showing cubic (400) and tetragonal (004) and (220) reflections. Trace from an as-fired surface of zirconia partially-stabilised with lime (CuKα radiation).

The transformation between tetragonal and monoclinic zirconia is believed to be martensitic[2]; it is fast and diffusionless. It is claimed that the tetragonal phase cannot be retained in bulk material to room temperature and atmospheric pressure by any quenching procedure[2,3]. Metastable tetragonal zirconia is known to exist at room temperature only in particles of less than ~ 30 nm diameter; in this case stability is due to the lower surface energy of the tetragonal phase compared with monoclinic[4] (although this explanation is not universally accepted[5]).

Our evidence for the existence of tetragonal zirconia in sintered bodies of lime-stabilised zirconia is extensive. We suspect that the magnesia PSZ material that has been reported to have high strengths[6] would show the same structural features. X-ray diffraction patterns have been obtained from foils thinned by an ion beam and unmachined surfaces, and may be clearly indexed as a mixture of cubic and tetragonal phases (Fig. 1). Transmission electron microscopy of these thinned samples reveals a duplex structure in which second phase particles up to ~ 100 nm in diameter are unambiguously tetragonal (Fig. 2). Carefully prepared samples show no evidence of the monoclinic phase unless the diameter of the second phase is > 100 nm. This conclusion is supported by thermal expansion measurements which exhibit no discontinuity on heating to 1,500 °C; a sudden change in length is normally a sensitive indicator of the presence of monoclinic zirconia (Fig. 3).

The tetragonal phase is metastable and can be transformed readily to monoclinic, for example by diamond grinding the surface or by pulverising during preparation for X-ray powder photographs. The stabilisation of the tetragonal phase is thought to arise from a combination of the surface energy effect discussed above and the constraint of the rigid matrix which opposes the transformation to the less dense monoclinic form[7]. As a result the transformation can occur locally once surface constraints are removed; it is probably triggered by shear strains. The transformation has been observed in thin foils during exposure to the electron beam in an electron microscope.

The presence of a tetragonal phase that can revert to the stable monoclinic form has a number of important consequences. The first is a significant increase in strength. Mean transverse rupture strengths of 650 MPa have been measured in lime-stabilised zirconia deformed in four-point bending. This strength is comparable with the highest values reported for oxide ceramics—in fine grained alumina[8]. By contrast the strength of PSZ containing a purely monoclinic phase is 250 MPa. The increase in strength is achieved by an increase in the work of fracture, up to 500 J m^{-2}, rather than by a reduction of defect size (usually grain size or porosity) as in many other strong ceramics. Part of the increased work of fracture probably arises from a dispersed phase interacting with the crack front, as discussed by Bansal and Heuer[9]. A more important contribution comes from the absorption of energy during the martensitic transformation of tetragonal particles to monoclinic (as in the TRIP steels[10]).

A further contribution to strengthening arises from surface finish. By contrast with other brittle materials, grinding the surface increases, rather than reduces, the strength compared with a polished finish. This difference is associated with an increase in the monoclinic content at the surface on grinding and is illustrated in Table 1. The proportions of the phases recorded there were estimated

Table 1 Influence of surface condition on strength and phase content (determined by X-ray diffraction) of zirconia partially stabilised with lime

	Transverse rupture stress (± s. d.) (MPa)	FWHM (111 cubic peak) (2θ)	% monoclinic at surface ({111} peaks)	% tetragonal at surface ({400} peaks)
As-fired	Not determined	0.22°	0	53
Diamond ground	600±11	0.46°	17	36
Ground and polished	490±30	0.35°	9–12	41
Ground and annealed for 1 h at 850 °C	500	0.36°	16	37

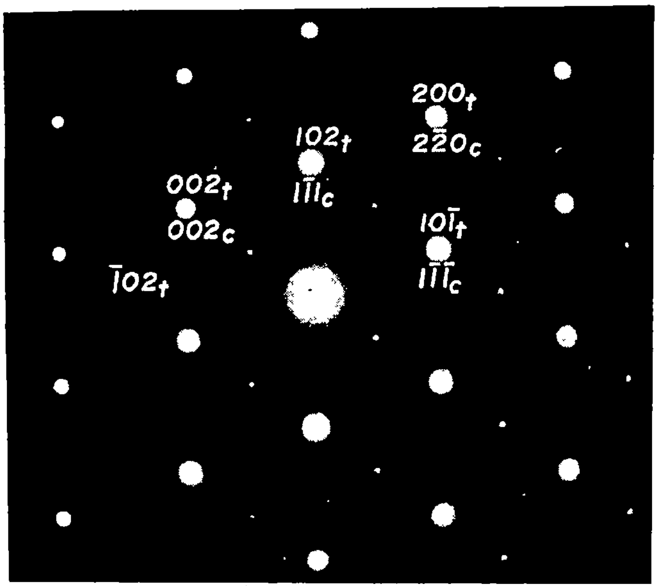
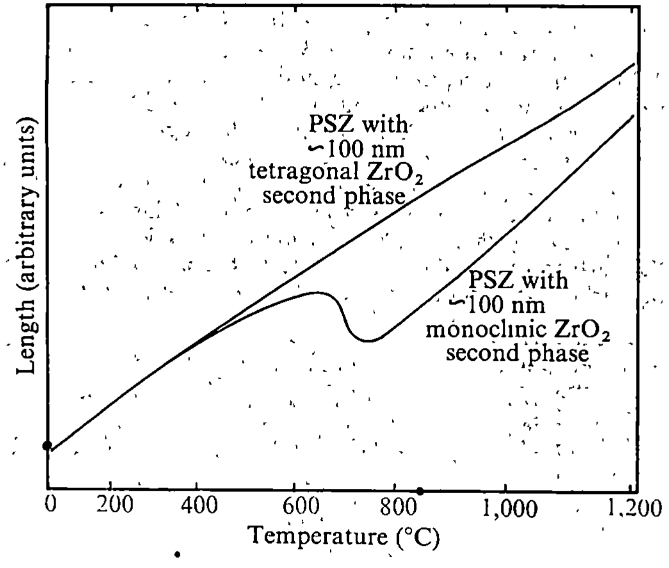


Fig. 2 Electron diffraction pattern of [110] cubic pole also showing tetragonal spots of [010]. Forbidden tetragonal spots occur due to double diffraction.

from the relative areas under the $(11\bar{1})_m$, $(111)_m$ and $(111)_c$ and the $(004)_t$, $(220)_t$ and $(400)_c$ (referred to as {400}) X-ray diffraction peaks[11]. An as-fired surface is free of the monoclinic phase, but this is generated (by transformation of the tetragonal phase) on grinding; this process induces compressive strains at the surface and increases the transverse rupture stress. Careful polishing removes some of the monoclinic material, without transforming any further tetragonal, and so reduces the surface strain and breaking stress. A similar reduction of strength can be achieved by annealing which removes the strain (indicated by FWHM) without reducing the surface content of monoclinic phase. Increases in the monoclinic content at the surface are matched by decreases in tetragonal content.

It may be noted that alloy systems based on zirconia have many features in common with systems based on iron, including three allotropes, martensitic transformations and metastable phases. The vast range of materials with very different properties formed in the iron system lead us to believe that our strong and comparatively tough alloys are but first developments in zirconia-based ceramics. We believe that, in development potential, PSZ can be considered a ceramic analogue of steel. Patents covering materials and processes have been applied for.

Fig. 3 Dilatometer traces obtained from PSZ with tetragonal and monoclinic second phases.



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Pulsating turbidity currents with relationship to high swell and high tides

THE Rio Balsas of Mexico has built a delta into the Pacific at latitude 18°N, virtually covering the narrow shelf, where the seaward slope is cut by numerous valleys. In 1967, during a period of large swell, Reimnitz[1] made a SCUBA dive to 18 m in a tributary of the Rio Balsas Canyon (Fig. 1) and encountered downcanyon current pulses estimated at more than 1 m s^{-1}. We visited the area on the RV Ellen B. Scripps in April 1975 and placed a current meter in Rio Balsas Canyon at a depth of 285 m. During the 5-d recording, a period of rather heavy swell occurred. This was indicated on our tide record (taken ~ 0.5 km inside the river estuary) by wave-induced oscillations with amplitudes up to 20 cm at periods of about 3 or 4 min. These persisted for approximately 36 h (Fig. 2). High swell was also observed on our fathometer records during traverses nearshore, and wave heights up to ~ 5 m were seen, considerably larger than during the rest of our study.

The computerised current meter record showing the times and velocities of upcanyon and downcanyon flows clearly indicates a change in the pattern during the period of high swell. Before and after the large waves, there was an alternation of flows up and down the canyon with velocities not exceeding 35 cm s^{-1}. The period of high swell, however, includes a downcanyon surge of 55 cm s^{-1}, followed by low currents lasting for 16 h, and then by six downcanyon surges all exceeding 50 cm s^{-1} (with two attaining 62 cm s^{-1}). These strong surges were each separated by intervals of ~1 h, during which there was no appreciable current, and seemed to be a type of low speed turbidity current. This contention is supported by our finding a coating of fine sand and silt on the case of the current meter that was positioned 3 m above the bottom.

A possible relationship of the surges to two high spring tides at that period should be considered seriously, in spite of the fact that the six closely spaced surges occurred somewhat before high tide. Tides in a narrow estuary or river

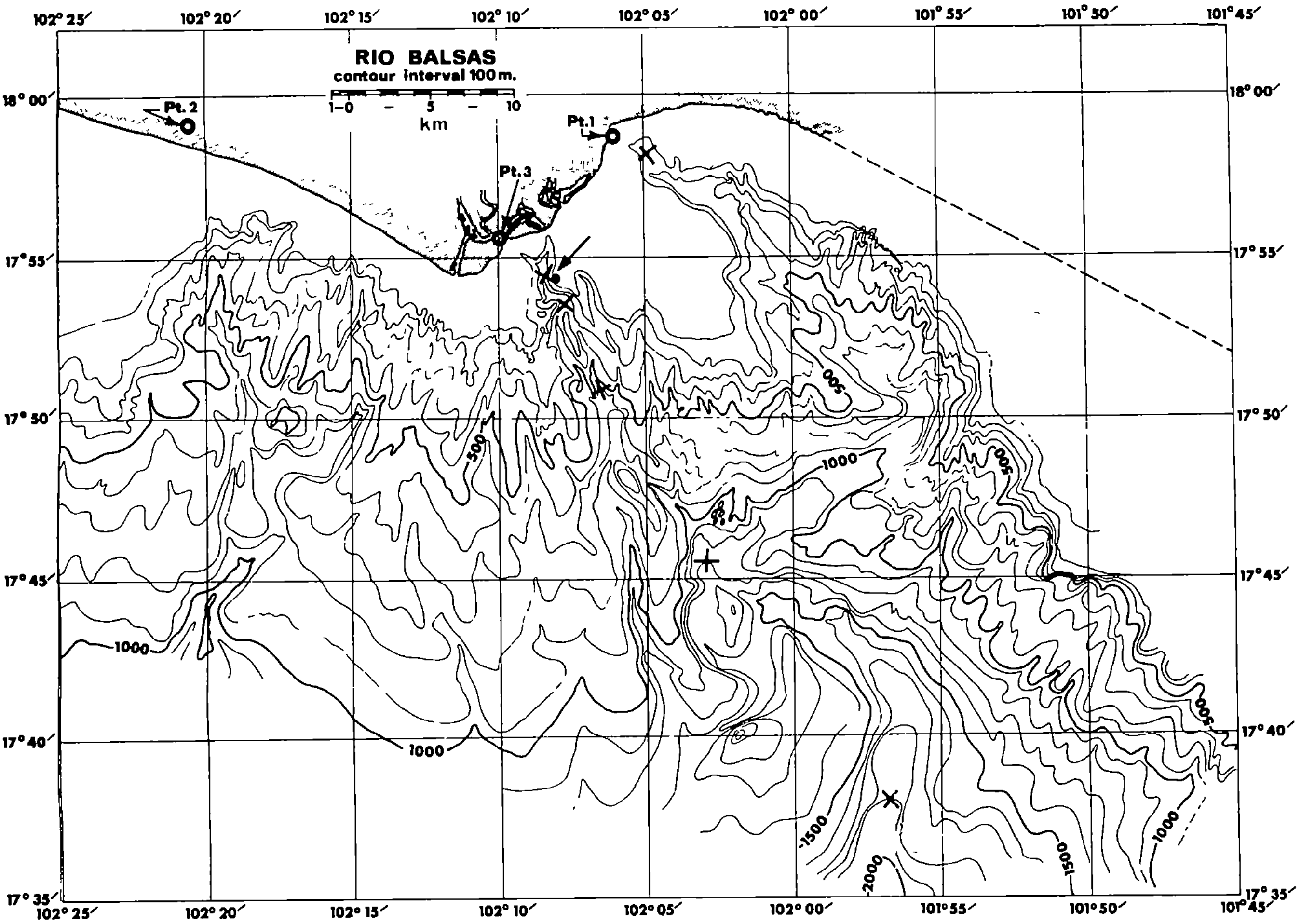

Fig. 1 The submarine valleys off the Rio Balsas Delta showing the location where the presumed turbidity current pulses were measured by a current meter (see arrow). Other crosses give locations of other current meters emplaced during the Rio Balsas Expedition.

mouth are always later than those on the open coast. Tide tables show many instances when the time difference is a matter of hours. If we assume that a difference of about 2 h existed here, the six surges could have occurred within the bounds of the second high tide. That this was actually the case is suggested by the fact that the time interval between the first large surge and centre of the six surges is very close to the interval between the two high tides.

Assuming that this relationship existed, it is possible to offer an explanation for the turbidity currents. Inman *et al.*[2] have found that storms with strong onshore winds cause water to set up along the shore, but with less rise at the head of a submarine canyon than over the adjacent shelf on either side. This imbalance develops a strong flow towards the head of the canyon and a return flow along the canyon. The same group also postulate that edge waves along the coast cause long period current oscillations that suspend sediment in the canyon heads, which, in storm conditions, may be capable of producing the density conditions necessary for turbidity currents with pulsating flows. On several occasions, we have had current meters carried down the canyons during these storm conditions at La Jolla, California[3]. Gennesseaux[4] also observed pulsating downcanyon flows off the Var River in Southern France during flood and storm conditions. Pulsating turbidity currents are, however, not necessarily due to storm conditions. Lambert and others[5] obtained a record of turbidity currents with pulsating flows of 30 cm s^{-1} in the Wallensee (in Switzerland) during heavy runoff from melting snows in the neighbouring mountains. Also, the pulsating flows observed by Reimnitz, mentioned previously, were during high swell rather than storm conditions.

Thus, it seems likely that large swell alone without high winds can produce a set-up of water along the shore and the necessary flow toward the canyon heads to produce pulsating turbidity currents. At the Rio Balsas Delta, it seems probable that the first strong downcanyon surge was developed only after the high tide had combined with the flows along the shore caused by the high swell. Then the rapidly falling tide prevented a recurrence until a considerable set-up of the water from the growing swell conditions was again favoured by the second rising tide, and the surges were able to repeat with only an hourly interval necessary for building up a head. After the sixth surge, the swell began to decrease and the falling tide helped bring the event to an end. It is significant that our current meter records, which were continued at somewhat greater depths in the same canyon head during the next week, showed no repeat of the strong surges, nor was there a repeat of either the large swell or of the high spring tides.

Although there is much evidence that turbidity currents can extend seaward for many miles, that may be true only during major high-velocity currents, like those of the Grand Banks earthquake[6,7]. There is evidence that the pulsating currents in the canyon off Rio Balsas did not extend any great distance. We had two other current meters in operation in the same canyon at distances of 17 and 36 km seaward of the point where the pulses were observed. Neither of these records showed any indication of unusual downcanyon flow during or after the period of large swell.

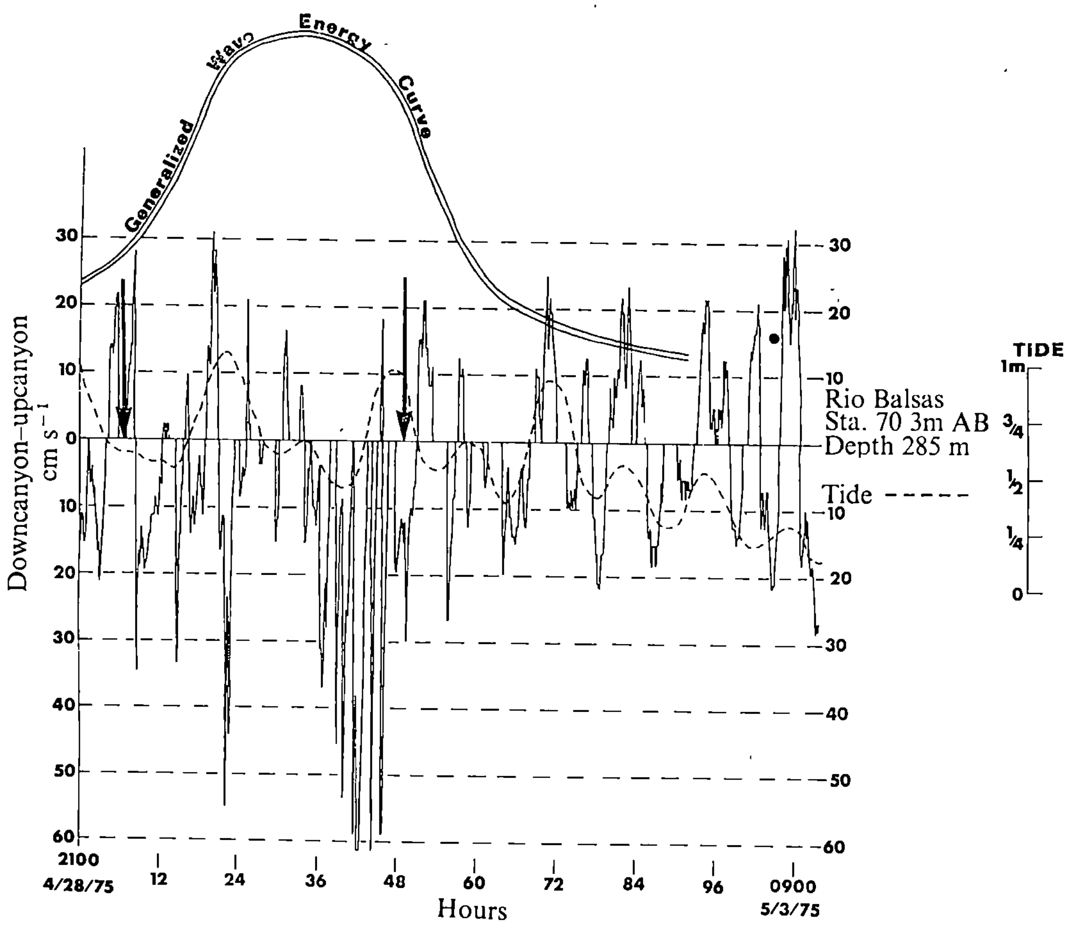

Fig. 2 The 108-h record of up- and downcanyon flows in the head of Rio Balsas Canyon at a depth of 285 m. Note the confinement of the large downcanyon surges to the period of large swell shown by the wave-energy curve. The two vertical arrows show the limits of large amplitude, short-period oscillations on the tide gauge inside the river mouth. The time of high tide at the gauge was probably appreciably later than at the canyon head.

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Hermatypic coral growth banding as environmental recorder

Study of incremental banding in coral skeletons was initiated by Ma[1] and later extended by Wells[2] and others[3]. More recently, discrete annual banding in the skeletons of certain hermatypic corals[4,5,6] has been described. Here we present an analysis of annual band width measurements from Bermuda corals which relates, through regression techniques, coral band time series to air temperature and air pressure variations. Our results indicate that coral bands record important aspects of their environment and therefore become useful where reconstruction of palaeoclimatic variables is of interest. Specifically the coral time series may be used as a palaeotemperature indicator or, when coupled with relatively well established palaeotemperatures, for palaeobarometric pressure determinations. Derived series of otherwise unobtainable palaeovariables are important not only for work on coral physiology, but also for construction and testing of climatic models; in this later instance information on palaeopressure is particularly desirable.

We chose Bermuda as a study area for a variety of reasons. Previous work[5] had revealed annual banding in the skeletons of the genus *Diploria*, correlations among individual coral heads, and low growth rates which allowed a large number of years to be recorded within a small coral. Geographically, Bermuda is at the northern limit of hermatypic coral distribution, providing an ideal site for the study of limiting factors of growth and for the monitoring of long term climatic changes. Because of Bermuda's near mid-oceanic position, the reconstruction of any climate variables at this site is of intrinsic meteorological interest.

Between May and June, 1973, 40 specimens of the brain coral *Diploria* species, ranging from 10 to 40 cm in height were collected in 2–5 m of water at various locations off the south and east shore of Bermuda (Spanish rock to the east of St Catherine's point). Specimen preparation and method of band measurement are described in Dodge and Thomson[5]. Briefly, coral sections were obtained and X radiographed to reveal annual bands. Six specimens were rejected because of small size (< 23 measurable bands), two because of non-hemispherical shape and heavy erosion by borers, two because of unclear annual bands and three because of faulty sectioning. Band widths for the 27 retained samples were measured and summarised into whole coral series in index form[7], which were then combined into a master chronology. Since banding patterns between individual heads were similar, summarising all corals into a master series maximised common variability yet minimised random and individual variations. Although measurable bands extended to the year 1900 AD in some corals, the number of individuals included in the chronology drop out at older times. To maintain the sampling standard deviation within a factor of 2 of the best sampled years, the series used in the regression analysis was terminated in 1905.

The choice of physical variables for comparison with the growth series was a compromise between availability of long records and known relationship to variables affecting corals (including water temperature, light, nutrition). Bermuda maximum air temperature[8] (1891–1966) and air pressure[9] (1880–1952) were considered the best available reflections of these influences. Water temperature is known to be well approximated by the former as shown by good agreement with short term recorded data (from the Bermuda Biological station, St George's, West Bermuda) and negligible island effect on surrounding climate[8], while light levels are hypothesised to be correlated with high pressure —which presumably makes for good weather and sunny days.

A 13-yr running average was applied to each series (Fig. 1*a*–*c*) to reduce the importance of poorly known short term coral growth dynamics[10], remove uncertainty in exact seasonal placement of band boundaries[5,11], and enhance long term climate fluctuations and coral response while minimising more random short term events. The overlap requirement for the three time series and the averaging procedure (which required dropping of six points at the ends) gave a 36-yr regression period (1946–11). When the coral band width chronology ($C(t)$) is regressed against maximal air temperature ($T(t)$) and air pressure ($P(t)$) (each series in index form), the result is

$$C(t) = -6.11T(t) + 3.12P(t) + 3.91$$

(significant by F test beyond the 0.01 level). The s.e. for the regression coefficient are 0.48 and 0.43 respectively. The multiple correlation coefficient $r=0.938$ (significantly different from zero beyond 0.01 level), so that the two variables explain 88% of the coral variance. The incremental contributions are 68% and 20% respectively. Figure 1*a* shows the coral series used in the regression and the coral series as reconstructed from the above equation The fit, while not perfect, is remarkably good and comparable in precision with fits of weather[12] and other[13] data obtained from tree-ring studies. The positive sign of the pressure coefficient reflects the expected relation of the corals to sunlight. The negative temperature coefficient, indicates increased growth (over the long run) during periods of lower than average water temperature. Although at first surprising, this becomes reasonable in the light of increased nutrient availability to corals during annual cold water overturn events[14]. The two coefficients thus offer a possible partial explanation of Bermuda coral growth: a negative relation to water temperature, which is actually a positive response to nutrient supply, and a positive relation to pressure, which may indicate a positive response to sunlight.

While the above results are useful in coral physiology studies, we would like to emphasise their utility in climate research where it is necessary to rely on historical weather records, often limited in extent, and where the introduction of information about additional climate variables or additional spatial locations would be particularly desirable Fortuitous agreement can never be strictly excluded without a detailed causal model of coral–climate interactions; however, the above results are statistically highly significant by conventional standards[11,13], and so offer the possibility of reconstructing additional palaeoclimatic variables from a variety of shallow oceanic locations. Since Bermuda maximum air temperature variations correspond well to global temperature variations[15] (see Fig. 1*d*), and since global palaeotemperatures are becoming increasingly common and refined through oxygen isotope and palaeontological data, a long coral growth series may be used to obtain other important variables, in particular palaeobarometric pressure. This information becomes available in oceanic areas where other biological sensors (for example tree rings) are not available. The length of the chronology of band widths may be greatly extended in time by sampling larger coral heads on the Bermuda platform and by cross-dating older fossilised heads which compose the reef framework[16].

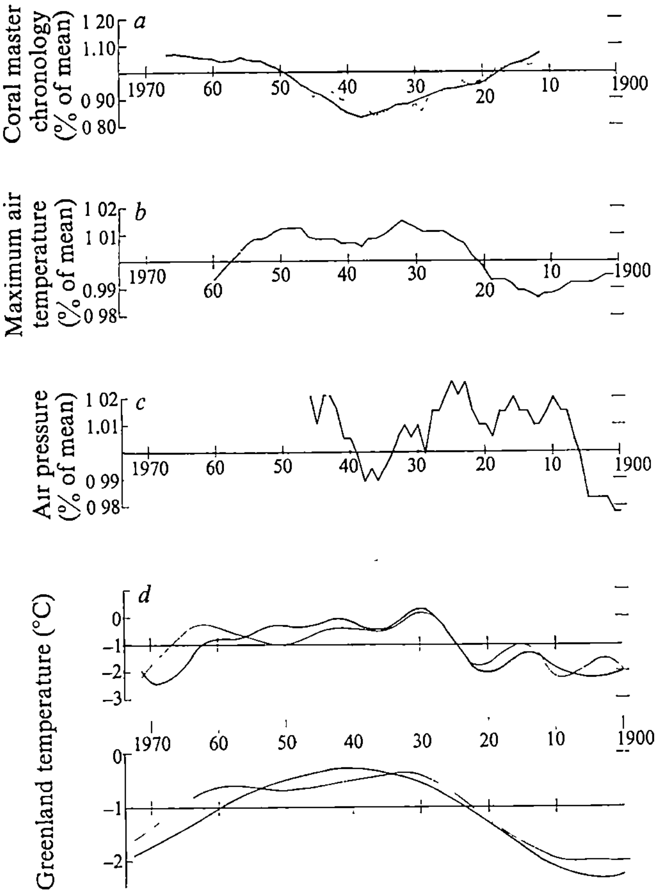

Fig. 1 *a*–*c*, Bermuda coral and climate time series smoothed by 13-yr running average. Data are presented as index values (each datum as a percentage of the particular series mean). The dashed line in Fig. 1*a* represents the coral series as reconstructed from the regression equation. Mean coral band width = 0.326 cm; mean maximum air temperature = 23.7 °C, mean air pressure = 18.7 mbar (+1,000 mbar) at sea level. *d*, From the data of Dansgaard *et al.*[15] Greenland temperature smoothed by 10 yr (top) and 30 yr (bottom) low pass digital filter. Climate changes are not simultaneous on the west coast (heavy line) and east coast (thin line).

Elsewhere long series of coral bands may be obtained at various past intervals (for example raised Pleistocene reefs) where, although overlap with the recent data may be impossible, fluctuations of past climate may be reconstructed In addition to their intrinsic value, long series of palaeovariables or their variations are potentially useful to meteorologists and climatologists who wish to incorporate boundary conditions for starting and running models or to test or verify models by comparing model prediction with reconstructed data.

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Intelligence tests may favour the majority groups in a population

DISPUTE over the observed racial differences in intelligence has centred on causal attribution: cultural bias in the testing, hereditary disparities between races and environmental variation. All systematic interpretations of these differences, however, rest on the same fundamental psychometric assumptions. We here examine one of those assumptions.

We have previously suggested that the existence of genetic environmental interactions[1] could affect item selection in the construction of intelligence tests using interaction in its precise technical sense in the analysis of variance. Most other available data disagree[2], but are correlational rather than experimental in nature. The correlation methods used have been suitable for detecting only certain forms of interaction: the mainstream of psychological thought on testing is strongly inclined to continuous models, which do not readily suggest nor encompass many kinds of interactions[3]. On the other hand, one could argue from genetic theory[4] that the number of possible interactions is so nearly infinite that no useful generalisations could result from pursuing such diversity. There has been almost no systematic attention to genotype–environment interactions in behavioural research or theory[5]. We here demonstrate that such interactions do in fact affect item selection and that these effects in turn contribute to the phenomena of group differences.

To reproduce minority group conditions several independent heterogeneous populations were formed from homogeneous groups, and intelligence tests were developed for each such base population using standard procedures. The characteristic performance of each group on each of these tests was then examined with reference to the representation of that group in the base population from which the test was derived. Hereafter 'test' refers to a test resulting from these test development procedures. 'Test base population' refers to the collection of individuals on which test development was based. 'Group' refers to a homogeneous group identifiable by common background. 'Level' refers to the level or extent of representation in the test base population.

The experimental plan was a Latin square of 6 rows of test base populations and 6 columns of levels of representation, with random cell assignments of 6 groups, subject to the constraints of Latin squares. The six levels of representation were 22, 15, 11, 8, 5, and 0 individuals. Groups were six homogeneous lines of rats randomly chosen from the writer's collection, for which detailed genetic and environmental conditions have been reported elsewhere[1]. The Latin square design, of course, provides that groups are randomly assigned in such a manner that each group appears once in each test base population and once at each level of representation. For the test development phase, individual animals were assigned on the basis of the Latin square. This established six independent test base populations each consisting of 61 individuals from each of the six groups at each of the six levels. To study characteristic performances, samples of 20 to 31 individuals were drawn from each of the six groups. Assignments of individual animals within the plan was random and blind: these were made by computer at the time of final data analysis.

Problems consisted of 19 geometric configurations of an extension of the Hebb–Williams maze[6,7], the conventional intelligence test for this species. Floors were either white or black, with partitions either of the same or contrasting colour. Thus four mazes were used, geometrically equivalent but differing visually; black on black, white on white, black on white, and white on black. One geometric configuration on one maze constituted one problem. Testing was at the rate of one problem a day with 15 trials per problem. Each geometric configuration was used in fixed order for four successive days, that is, over the four mazes, with order assigned at random for each individual for each configuration. Five measures were taken on each problem: mean start delay, mean exit delay, mean run time, trials to criterion, and number of errors. In all, 380 performance measures or item scores were obtained.

For the test development phase standardised item scores and test scores (the total of item scores) were used. Standardisation was based on the pooled data for all individuals. Correlations were obtained betweem item scores and test scores on each of the six test base populations independently. The 50 items showing highest correlation with the test scores for each test base population were then selected as an intelligence test for that population. This is, of course, the conventional procedure for item selection in test construction and development.

For the characteristic performance phase each of the resulting six tests was then 'administered' to the individuals in the characteristic performance samples drawn from each of the six groups. For each group independently this provided a mean or characteristic performance score on each of six different tests. From the Latin square each group was represented at a different level in each of the six test base populations from which the tests were derived. For each group both the characteristic performance means on the six tests and the level of representation of that group in the corresponding test base populations were ranked. For each group the rank difference correlation between rankings shows the relationship between group means and level of representation in the base population. The average of the six correlations was 0.562. The mean of the corresponding normal deviates from the tabled exact distribution yielded $z = 3.33$, $P < 0.001$ (ref. 8).

The mean characteristic performance of homogeneous groups was found to vary directly with the relative numbers of that group represented in the base population from which the test was derived. Therefore, there is a test bias inversely proportional to representation in the base population.

The results confirm the interaction hypothesis, and, moreover, it is now clear that genetic–environmental interactions do indeed affect item selection under the procedures commonly used in test construction. It should be emphasised, however, that these results and their implications stand independently of any heredity–environment controversies. The psychometric implications would be identical had the homogeneous group differentiation been based on controlled environmental rather than genetic variation. Indeed, it seems reasonable to expect subsequent research will demonstrate interactions also occur with such environmental differentiation.

Finally, generalisation from these data to man is direct and not analogical: the experiment was an empirical test of common psychometric assumptions and procedures. Generalisation is therefore to those assumptions and procedures. The implications are far ranging. Majorities will score higher than minorities as a general artefact of test construction procedures. Theoretical approaches which ignore the existence of genetic–environment interactions ignore a significant source of individual and of group variation.

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Radiocarbon date for the largest extinct bird

IN December 1973 two fragments of an eggshell of the extinct giant elephant bird, *Aepyornis* sp., were obtained from a site 50 km west south-west of Fort Dauphin (21°1′S, 47°E), southern Madagascar. The bird, similar in appearance to, but not directly related to, the ostrich (*Strathio camelus*) was the largest ever to exist; the largest species had a height of 3 m, and laid eggs up to 35 cm long[1]. The site from which the small fragments were obtained is located in coastal sand dunes some 100 m from the shoreline. The immediate region is underlain with granitic rocks capped by igneous detritus. The climate is semi-arid with a mean annual rainfall of 500–600 mm. The vegetation consists of bush interspersed with many endemic succulents including the very tall, stalk-like plants of the Didiraeceae family[2].

We have obtained a radiocarbon date from one of the shell fragments, about 4 mm thick and weighing 11.78 g. To remove surface contamination the outer layer of the shell was removed with acid. The interior of the shell was then dissolved in hydrochloric acid and the resulting CO_2 carefully purified to exclude electronegative impurities. The $^{14}C/^{12}C$ ratio was determined in a CO_2 proportional counter to a statistical accuracy of 1σ standard deviation. An aliquot was analysed in a mass spectrometer, and its $^{14}C/^{12}C$ isotopic ratio was found to be $-14.44‰$ with respect to Craig's PDB standard. The radiocarbon age was calculated to be 1,000 ± 150 yr (UCLA—1893), which includes a correction for isotopic fractionation.

Tree-ring calibration of this date shows that the true age lies well within the margin of error[3]. Other egg shells have been dated using radiocarbon determination and have been found to give reliable dates[4]. Little contamination is to be expected from the arid environment containing little carbonate, except for that from sea shells in the sand dunes. Moreover, the removal of the outer shell layers of the *Aepyornis* shell fragment reasonably ensures that the date is not significantly in error.

An egg fragment found in sand dunes 3 km north-east of the village of Irodo, 45 km south-east of Diego-Suarez (12°19′S, 49°17′E) on the northern tip of Madagascar, has been radiocarbon dated at 1,150 ± 90 yr old, and nearby, an archaeological site has been dated at 1,090 ± 90 yr (Gak—692), (refs 5 and 6). Moreover, a number of egg fragments collected in locations around southern Madagascar have been dated at 1,970 ± 90, 2,930 ± 85 and 5,210 ± 140 yr old[7,8]. It is especially noteworthy that in the archaeological site of Talaky on the southern coast of Madagascar (25°19′S, 45°30′E) *Aepyornis* egg shell fragments were found mixed in with pottery and other artefacts, including iron fragments. This site has been dated at 840 ± 80 yr (Gak—276). A historic reference by Flacourt[10] even implies that these large birds may have existed in remote areas of Madagascar as late as the 17th century. Other archaeologic sites are known to contain undated *Aepyornis* egg shell fragments.

The radiocarbon date presented here is of essentially the same period as an egg from the extreme north, suggesting that these birds were still widespread as late as the 10th century AD. It is thought that man entered Madagascar only as late as the middle of the first millennium AD[11] and it is possible that the fate of the giant bird population is tied to the late arrival of man. Yet the extinction of the elephant birds apparently occurred over many centuries and not suddenly. It is uncertain to what extent forest clearing played a major role in denying the birds a protective habitat.

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Scorpionflies as kleptoparasites of web-building spiders

ONLY a few species of spiders and insects are known regularly to inhabit spider webs. Known associations involve predation or commensalism. Spiders of the family Mimetidae invade the webs of other spiders to devour the owner of the web. Small spiders of the genus *Argyrodes* (Theridiidae) are often seen in the webs of larger spiders, where they seem to consume only small insects ignored by the spiders that constructed the web; this relationship is thus commensalism and not parasitism[2]. The insect *Ranzovius* (Hemiptera: Miridae) seems to be a commensal in the webs of certain spiders[3]; however, *R. fennahi* may be predaceous on spider eggs[4]. The tropical hummingbird, *Phaethornis superciliosus*, has been observed feeding on insects

Table 1 Observed kleptoparasitism of spiders by panorpas

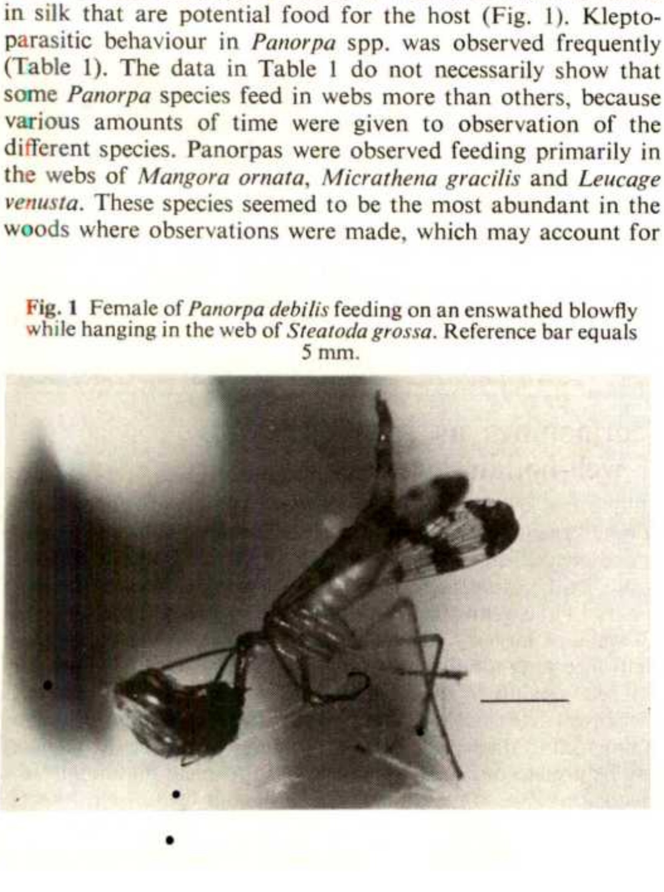
	No. of webs parasitised							
Panorpa sp.	*Agelenopsis* Agelenidae	*Theridion frondeum* Theridiidae	*Mangora ornata* Araneidae	*Leucauge venusta* Tetragnathidae	*Micrathena gracilis* Araneidae	*Argiope aurantia* Araneidae	Unknown Spiders	Row totals
banksi	1	3	8	4	5	1	3	25
claripennis	0	2	5	2	6	0	6	21
debilis	0	2	6	9	10	1	2	30
helena	2	0	3	2	3	0	0	10
latipennis	0	0	2	2	1	0	4	9
maculosa	0	0	1	0	1	0	0	2
mirabilis	2	1	7	4	4	0	10	27
nebulosa	0	0	5	4	1	0	5	15
submaculosa	0	0	4	3	3	2	2	14
Column totals	5	8	41	30	33	4	32	153

Percentage kleptoparasitic feeding by each species (number of observations of feeding in spider webs per total number of observations of feeding on dead insects) was: *P. banksi*, 30.1; *P. claripennis*, 29.2; *P. debilis*, 30.3; *P. helena*, 15.6; *P. latipennis*, 11.7; *P. muculosa*, 4.77; *P. mirabilis*, 31.0; *P. nebulosa*, 36.6; *P. submaculosa*, 22.2. Percentage kleptoparasitic feeding for all species combined was 24.6%.

trapped in webs of *Nephila clavipes* (Araneidae). The hummingbird–*Nephila* association may be parasitism because although only very small insects are taken by hummingbirds, they may remove a portion of the host's web for nest material[5]. I report here the kleptoparasitism of the prey of web-building spiders by scorpionflies of the genus *Panorpa* (Panorpidae), and I consider a likely selective context for the evolution of this unusual feeding strategy in scorpionflies. Field observations were conducted in south-eastern Michigan near Ann Arbor during 1971–74 (ref. 6).

Panorpas are scavengers, feeding primarily on soft-bodied dead or moribund insects, but occasionally eating living slugs, vertebrate carrion and pollen. Dead insects comprised between 89 and 97% of the diets of the nine species of *Panorpa* that occur in south-eastern Michigan. Diptera made up 47–69% of the dead insects in the diets of the species. A total of 675 feeding observations on the following nine species of *Panorpa* were recorded: *banksi* (86 observations), *claripennis* (75), *debilis* (102), *helena* (67), *latipennis* (87), *maculosa* (45), *mirabilis* (98), *nebulosa* (44), *submaculosa* (71). (See ref. 3 for details of food items and feeding behaviour of panorpas.) Panorpas are true kleptoparasites of web-building spiders, that is they enter webs and feed on trapped insects as well as on insects swathed in silk that are potential food for the host (Fig. 1). Kleptoparasitic behaviour in *Panorpa* spp. was observed frequently (Table 1). The data in Table 1 do not necessarily show that some *Panorpa* species feed in webs more than others, because various amounts of time were given to observation of the different species. Panorpas were observed feeding primarily in the webs of *Mangora ornata*, *Micrathena gracilis* and *Leucage venusta*. These species seemed to be the most abundant in the woods where observations were made, which may account for the large number of observations of panorpas parasitising these particular spiders.

Fig. 1 Female of *Panorpa debilis* feeding on an enswathed blowfly while hanging in the web of *Steatoda grossa*. Reference bar equals 5 mm.



A panorpa, when it locates a spider web, may either fly directly to and land on an trapped insect, or walk into the web from the adjacent vegetation. Panorpas usually feed in the web and only leave it when approached by a spider; however, *P. banksi* on three occasions, and *P. helena* on one occasion, removed muscoid flies from the webs of *M. gracilis* and flew out of the webs with the flies in their mandibles. Panorpas move around in spider webs with little difficulty, though occasionally they may become entangled temporarily. If they cannot pull themselves free, they regurgitate a brown enteric fluid on to the portion of the web trapping their appendage(s). This fluid originates in the midgut and is similar in appearance to the tobacco juice of grasshoppers. Visual observations revealed that the webbing that traps panorpas dissolves when flooded with this secretion. Five *P. submaculosa* and five *P. banksi* were artificially entangled in the webs of *M. gracilis* in the laboratory to determine how rapidly they could free themselves; they escaped from the webs after 10 s–2 min ($\overline{X} = 1.1$ min). Seven milked *P. banksi* (hand-held until they no longer regurgitated on my fingers) were unable to escape subsequent entanglement in webs of *M. gracilis* after 30 min.

Panorpas readily enter webs occupied by spiders. *Mangora* spiders are usually too small to capture and kill panorpas; however, *M. gracilis*, *L. venusta*, *Argiope aurantia* and some of the undetermined spiders (families Araneidae, Dictynidae and Theridiidae) do prey on panorpas. Fifty-nine per cent (53 observations) of the mortality of panorpas observed during 1971–74 was by web-building spiders. All the nine spiders of *Panorpa* studied, except the very large and aggressive *P. mirabilis*, were found in spider webs. The remains of equal numbers of male and female panorpas were seen in webs[6].

When a panorpa feeding in a web is approached by the web owner, it may fly or drop from the web, or it may behave aggressively toward the spider. *Panorpa* males are armed with an enlarged genital bulb terminating the mobile abdomen, which is used in aggression towards spiders as well as towards other panorpas encountered around food. When a spider is struck by this genital bulb is usually stops briefly and may retreat. A *Panorpa* female, on the other hand, has no bulbous external genitalia, but may strike a spider with her abdomen, or butt it with her head. Aggressive behaviour of panorpas usually deters attacks by the small spider *M. ornata* and by the larger *L. venusta* and *M. gracilis*. Six *P. submaculosa* (three males and three females) and five *P. banksi* males were observed to act aggressively towards attacking *M. gracilis* in nature. The spiders were deterred and the panorpas escaped.

When a panorpa is seized by a spider, the panorpa regurgitates the same brown enteric fluid mentioned earlier and attempts to smear this on the predator. When applied by panorpas, the fluid was found to be an effective deterrent against the web-building spiders tested (*M. ornata*, *L. venusta* and *M. gracilis*). Ten individuals of *P. debilis* were used to test the effectiveness of the enteric fluid against predation by spiders. The panorpas were introduced into pint jars containing spiders which had been starved for 3 d. Only two of the ten panorpas received eventually fatal injuries from the bite of a spider. Eight milked individuals of *P. debilis* were similarly tested and all fell prey to the spiders. When spiders are dabbed with the enteric fluid by panorpas they desist and begin cleaning themselves. The enteric fluid applied by panorpas is also effective against wolf spiders, crab spiders, jumping spiders and carpenter ants. The oral effluent of saw-flies, which consists of terpenoid resins sequestered from the host plant, is an effective deterrent against insect and spider predators[7]. The brown fluid regurgitated from the crop by grasshoppers also seems to have a protective function[8].

Scorpionflies are common over much of eastern North America and are typically inhabitants of the herb strata of moist deciduous forests. In such habitats they may be the most abundant large insect. Adults of several species of *Panorpa* may occur together, often in large numbers, in the same woods. A 3-yr study of nine species of *Panorpa* in south-eastern Michigan reveals that the species overlap almost completely in their feeding ecology and that food is a limiting resource for these insects[6]. The species are not separated with respect to habitat utilisation, feeding location or time, or food type, however, the timing of adult seasonal emergence reduces overlap among the species. The *Panorpa* species investigated compete primarily in an interference manner (aggression) through intense intra- and interspecific aggression around food. Panorpas also share food with certain other arthropods (for example phalangids and scavenger beetles). Interference competition for food in panorpas influences the fitness of the competing individuals, since increased competition in panorpas results in reduced adult longevity under natural conditions. The reduction in longevity is a result of increased dispersal and movement into less desirable and more risky habitats in terms of exposure to predators. A selective history involving intense competition for food could account for the evolution of the dangerous strategy of kleptoparasitism of web-building spiders by panorpas.

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Natural daylengths regulate insect seasonality by two mechanisms

FOR many animal species in temperate regions of the world, the photoperiod is the major environmental factor that reliably signals forthcoming seasonal changes. With the exception of studies dealing with several species of birds[1,2], experiments generally have not shown what features of the natural photoperiod are used by animals to perceive calendar time. Not only have investigations of photoperiodicity usually used stationary photoperiods in the laboratory, but generally they have not taken into account the seasonal variations in the rate of change in the length of the day[1]; these changes are greatest at the time of the equinoxes and smallest at the time of the solstices. Our results demonstrate for the first time that insects have evolved at least two mechanisms based on seasonal changes in photoperiod to end diapause at the end of winter.

We investigated three possible mechanisms for this. First, we considered whether the insects perceive day (or night) length as being either long or short; that is, day (or night) lengths shorter than a critical photoperiod elicit one response, and days (or nights) longer than a critical photoperiod elicit an alternative response. For example, in some insect species one or two critical photoperiods control diapause induction[3–6]. There is, however, evidence from only two species, *Nemobius yezoensis* Shiraki[7] and *Wyeomia smithii* (Coquillett)[8], that diapause termination is also controlled by critical photoperiods.

Second, we considered whether the insects perceive the actual duration of photophases (or scotophases) and show a quantitative (graded) response to seasonally changing daylengths One insect species, *Chrysopa carnea* Stephens, has been shown to maintain diapause primarily through a quantitative response to the absolute duration of the shortening autumnal daylength and the very short days of early winter[9]. Quantitative responses to the duration of stationary daily photoperiods have also been shown in the laboratory for Odonata[10,11] and for Diptera[12]. There is no evidence, however, that this mechanism is used by insects to terminate diapause.

Third, we considered whether the insects perceive and respond to the direction of daylength changes *per se*, regardless of the actual daylength. Although *C. carnea* can perceive and respond to the direction of large daylength changes in the laboratory[13], it has not been shown conclusively that insects react to the direction of naturally changing daylength.

Our results, derived from experiments with two species of green lacewings (Neuroptera: Chrysopidae), show that in nature, insects use at least the first two of the above three mechanisms in timing the end of their winter dormancy. In one species, *Meleoma signoretti* Fitch, days (or nights) are perceived as either long or short, and diapause ends when late winter days exceed a critical length (the first mechanism). In the other species, *Chrysopa downesi* Banks, diapause terminates at a rate directly related to lengthening days (the second mechanism)

Both of these lacewing species are, in nature, univoltine (they have one generation per year) and they spend a large portion of their annual cycle in an aestival–autumnal–hibernal diapause which is induced, maintained, and terminated primarily by photoperiod[14]. Therefore, to determine the type of photoperiodic stimuli that end diapause, we sampled natural populations from late autumn through to early spring. All test animals were F_1 offspring of females collected at Ithaca, New York. Individuals were reared and maintained outside according to methods given previously[15]. At each sample date we transferred 10–20 individuals into each of several stationary photoperiodic regimes and into a heated greenhouse under natural illumination (Tables 1 and 2).

The results (Table 1) for *M. signoretti* show that: first, diapausing larvae transferred to long day regimes in the laboratory terminate diapause within a relatively narrow time period, or they remain in diapause until death, if transferred to short day regimes. Second, natural daylengths shorter than a critical photoperiod maintain diapause during autumn and most of winter. Third, near the end of winter, natural daylengths which exceed the critical photoperiod provide the primary stimulus for diapause termination. Fourth, although the intensity of diapause decreases as the season progresses, the critical photoperiod does not change substantially, and the insects' response to photoperiod ends abruptly after diapause terminates, and fifth, the critical

Table 1 Median date of diapause termination (larval–pupal ecdysis) by *Meleoma signoretti* after transfer from out doors to various photoperiodic regimes

Date of transfer	Photoperiod (LD) 10:14	12:12	14:10	16:8	Natural
Nov. 22	— (0)	— (0)	Feb. 1 (10)	Feb. 1 (10)	Apr. 26 (2)
Dec. 22	— (0)	— (0)	Jan. 13 (11)	Jan. 31 (9)	Apr. 28 (5)
Jan. 22	— (0)	— (0)	Feb. 9 (10)	Feb. 9 (8)	May 1 (8)
Feb. 22	— (0)	— (0)	Mar. 5 (11)	Mar 7 (12)	Apr. 20 (7)
Mar. 22	Mar. 30 (12)	Mar. 30 (12)	Mar. 30 (12)	Mar. 30 (12)	Mar. 31 (12)

Temperature: stationary photoperiods 24 ± 1 °C; natural daylength 23 ± 4 °C. No of surviving individuals, from the original 12 to 14, in parentheses.

photoperiod for diapause termination in the laboratory is between light–dark (LD) 12 : 12 and LD 14 . 10. This range in photoperiods corresponds with the daylength (sunrise to sunset, plus twilight) in March, at the time diapause ends in nature.

The higher incidence of pupation in late season samples (Table 1) suggests that low temperatures enhance survival during diapause, especially at the end of winter when metabolic reserves are probably low. The approximately one-month delay in pupation in the November, December, January and February samples (Table 1), for natural daylength (in relation to the March sample) suggests that, although low temperatures are not necessary for ending diapause, they hasten diapause termination at the end of winter.

Table 2 Number of days (mean ± s.d.) to terminate diapause (initiate oviposition) by *Chrysopa downesi* after transfer from outdoors to various photoperiodic regimes

Date of transfer	Photoperiod (LD) 11:13	12:12	13:11	14:10	15:9	Natural*
Dec. 22	‡	78±12	22±5	17±5	14±2	92±10
Feb. 7	81±12	46±25	10±3	—	8±3	32±5
Mar. 1	40±34†	7±1	7±2	—	—	6±1
Mar. 21	4±2	4±1	3±1	—	—	4±1

Temperature: stationary photoperiods 24±1 °C; natural daylength 23±4 °C. Number of pairs in each condition 8 to 10; incidence of oviposition in each condition 80 to 100%.

*Median dates of diapause termination in the December 22 to March 21 samples are April 5, March 20, March 8 and March 25 respectively.

†Diapause had broken in two animals in this sample.

‡Death occurred before diapause termination.

In contrast to those with *M. signoretti*, the tests (Table 2) with *C. downesi* show that: first, diapausing adults transferred into the laboratory after the winter solstice exhibit a quantitative response to daylengths that lie between LD 11 : 13 and LD 15 : 9, that is in the December 22 and February 7 samples the rate at which diapause comes to an end is directly related to the actual duration of daylength. Second, diapause maintenance after the winter solstice is related to the actual duration of the short winter days. Third, diapause is gradually brought to an end by the lengthening late winter days, that is, as the days lengthen there is an increase in the rate of diapause development, and diapause ends near the time of the vernal equinox (between March 1 and March 21), and fourth, diminution of both diapause intensity and sensitivity to photoperiod, which occurs slowly at the beginning of winter and quickly at the end of winter, is directly related to actual daylength.

Thus the data indicate that *C. downesi* and *M. signoretti* have evolved grossly different photoperiodic reactions that serve to synchronise the end of their diapause with the beginning of spring. In the case of *C. downesi* and other *Chrysopa* species for which we have considerable phenological data, the type of response is consistent with the phylogenetic relationships of the species[16]. For example, comparisons between populations of *C. downesi* and the closely related *C. carnea* from the north-eastern USA, show that both respond quantitatively to actual daylengths during diapause maintenance but each exhibits the response at a different time of the year—autumn for *C. carnea* and early and late winter for *C. downesi*. As a result, these sympatric species, which readily hybridise in the laboratory and which hibernate in overlapping habitats, end diapause and begin postdiapause reproductive activity at least a month apart. This seasonal difference in the timing of reproduction helps prevent the univoltine *C. downesi* from hybridising with its close relative *C. carnea*. Therefore, the characteristic response of *C downesi* to daylength during the winter not only serves to time the end of diapause with the advent of favourable spring conditions, but it also functions as an effective reproductive barrier which maintains this species as a separate entity in the regions of north-eastern USA and eastern Canada.

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Effect of light on egg-laying rate and mating speed in phototactic strains of *Drosophila*

IN *Drosophila*, phototactic behaviour can be quantitatively described using Hirsch–Hadler classification mazes[1]. Flies entering the maze make a series of 15 consecutive light–dark choices and receive phototactic scores ranging from 1.0 (highly photonegative) to 16.0 (highly photopositive). A photoneutral population has an expected mean photoscore of 8 5. The phototaxis maze has provided a useful tool for the characterisation of phototactic behaviour in natural populations of *Drosophila* and for the creation of photopositive and photonegative strains of flies by artificial selection Natural populations of *D. melanogaster* and *D. pseudoobscura* are photoneutral in general, but respond rapidly to selection for positive or negative phototactic behaviour[2,4,7,8]. Genetic analysis of phototaxis in *Drosophila* suggests that this behaviour is a polygenic trait with low heritability[3,4,6]. Suspension of artificial selection results in rapid reversion to photoneutrality which illustrates the principle of genetic homeostasis and suggests that changes in fitness accompany selection for photopositive and photonegative behaviour[8].

Two components of fitness—rate of egg deposition and mating speed—were measured in an unselected population and in a photopositive and photonegative population of *D. melanogaster*. The investigation was carried out under two sets of environmental conditions: continuous darkness and continuous light. Ten pairs of flies from each population were maintained separately for 1 week in continuous light and ten pairs from each population were maintained for 1 week in continuous darkness. Five replications were performed for each population in each condition. Egg-laying surfaces containing fresh cornmeal–molasses medium were replaced daily and the number of eggs deposited during the preceding 24 h was recorded.

Table 1 Average number of eggs laid by phototactic strains of *D. melanogaster* during 1 week of constant light and constant darkness

Population	Light (mean ±s e.)	Dark (mean ±s.e.)	*t*
Unselected (7.9±0.12)	1,111.2±27.61	1,110.1±34.76	0.25
Strain 0 (+) (13 93±0.14)	748.2±33.11	526.6±25 26	5 32*
Strain 0 (−) (2.91±0.11)	560.4±36 17	1,672.7±126.9	15.22†

* $P = 0.005$.
† $P = 0.0001$.

Results are shown in Table 1. The unselected strain was fairly photoneutral and the number of eggs deposited during 1 week did not differ in light or darkness. On the other hand, strain 0 (+) was highly photopositive and is seen to lay more eggs in continuous light than in a continuously dark environment. The opposite is true of flies from strain 0 (−), many more eggs being deposited when the flies were kept in continuous darkness.

To determine whether reduced insemination in light or dark was responsible for the reduction in oviposition, the effect of light and darkness on mating speed was investigated among flies from each population. Groups of twenty virgin males and twenty virgin females from each population were stored separately at 24±1 °C for 3 d. Males and females from the same population were then placed together in total darkness or light. After 5, 15, 30 and 60 min females were removed and placed individually in vials. At least five replications were made of each test. The presence of larvae in the vial indicated that the female had mated.

The proportion of flies mating at each time is shown in Fig. 1. In the unselected population, flies mated faster in the light. After 2 h (not shown) virtually 100% of the females had mated. Flies from strain 0 (+) mated even faster in the light than did the unselected flies. At all four times, however, a greater proportion of flies from strain 0 (−) had mated in darkness than in the light. Early mating was definitely inhibited by the unpreferred environment but within 1 or 2 h females from all strains had mated both in light and in darkness. Therefore absence of insemination is not likely to be the cause of the alteration of oviposition rates in phototactic strains when placed in an unpreferred environment.

Table 2 Average number of eggs laid by phototactic strains of *D. psuedoobscura* during a week of constant light and constant darkness

Population	Light (mean ±s.e.)	Dark (mean ±s.e.)	*t*
Unselected (8 6±0.19)	834.6±112 94	880.6±69.44	0.34
Strain 25 (14.93±0.08)	1,641.0±66.18	868.0±115.14	5.82†
Strain 27 (2.54±0.10)	1,346.6±81.94	2,026.2±152.35	3.92*

* $P = 0.005$.
† $P = 0.0005$.

The effect of light or dark on egg deposition was examined in a photopositive population (strain 25) and a photonegative population (strain 27) of *D. pseudoobscura* obtained from Professor Th. Dobzhansky. A recently collected population of *D pseudoobscura* was provided by Dr F. Ayala. Egg deposition was measured in these three populations by the same method described for *D. melanogaster* (Table 2). Again, the presence or absence of light had no effect on the number of eggs laid by the unselected flies. Continuous light or darkness affected the photopositive and photonegative populations of *D. pseudoobscura* in the same way as observed for *D. melanogaster*.

It is difficult to know whether the preferred environmental condition (light or its absence) stimulates oviposition or if the unpreferred condition inhibits egg laying, or both. Regardless of the cause, it is obvious that the *Drosophila* species investigated here have a greater relative fitness in environments that they are genetically influenced to select.

Both *D. melanogaster* and *D pseudoobscura* are light-independent with respect to mating behaviour[9]. Our results show that selection for phototactic behaviour may modify this light-independence in one direction or the other, at least for early matings. Photopositive and photonegative strains of *D. pseudoobscura* have been shown to mate with similar frequency in light, while in darkness, photonegative flies mate slightly more rapidly[10].

Equally important to overall reproductive fitness is the

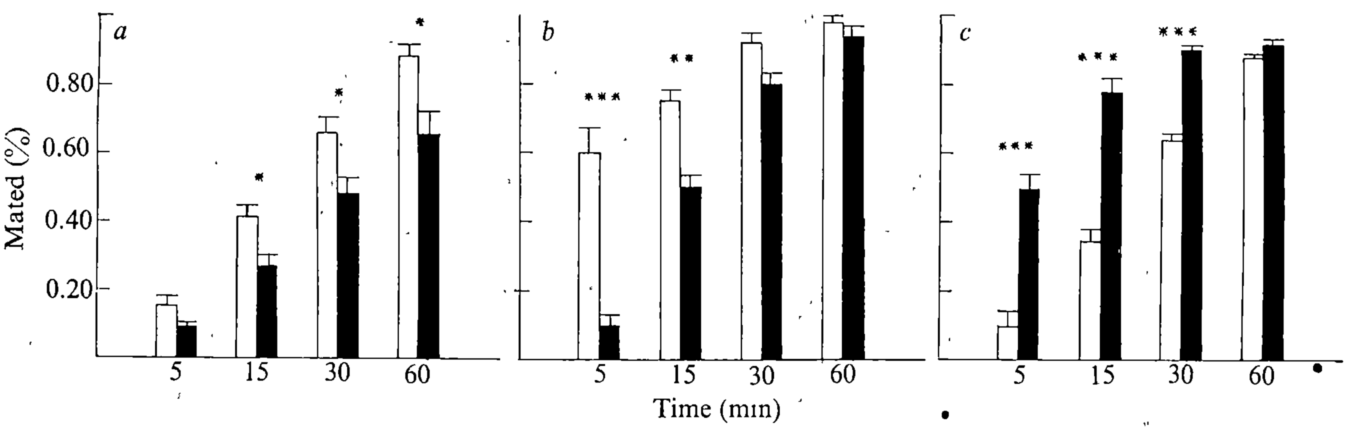

Fig. 1 Average percentage of females mated after 5, 15, 30 and 60 min in light (open bars) and darkness (solid bars) *a*, Unselected; *b*, strain 0 (+); *c*, strain 0 (−). * P=0.05; ** P=0.01; *** P=0.005.

deposition of eggs after insemination. Individuals mating more slowly and depositing fewer eggs will leave fewer offspring relative to those individuals that mate and oviposit quickly. Light is an important environmental variable affecting natural populations. The presence of genetic variation for phototactic behaviour may be extremely important for adaptation of *Drosophila* populations to new or changing environments. In the experiments reported here, the light-dependent fitness of flies is directly related to their genotype for phototactic behaviour. Mayr[11] has proposed that behavioural changes precede morphological changes during evolution. The results presented here strongly support his idea.

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Corpus allatum activity and wing determination in *Megoura viciae*

ALARY polymorphism in aphids is influenced by several environmental stimuli acting prenatally and/or postnatally[1]. In *Megoura viciae*, alatae are determined prenatally by exposing previously isolated apterae to a short crowding stimulus[2], and the physiological mechanism controlling this process would be expected also to show an immediate response to the stimulus. White[3] suggested that the prenatal physiological control of alary polymorphism is exercised by the activity of the maternal corpus allatum (CA) which would produce less juvenile hormone (JH) when aphids are crowded than when they are isolated. She assumed that CA volume and nuclear diameter were directly proportional to JH synthesis and secretion and she reported that in *Brevicoryne brassicae* the CA nuclear diameter is greater in isolated aphids than in crowded aphids[3]. She also found that CA volume was greater in alatae, which have only apterous progeny, than in apterae which have alate and apterous progeny[4]. The application of JH analogues to alatiform larvae resulted in the apparent suppression of alate characters[5,6]. Experiments with aphids, however, have shown that synthetic JH only initiates the production of supernumary larvae[7] and that it has no apterising effect[8]. In locusts volume cannot be correlated with JH concentration in either CA or haemolymph[9]. These studies have raised serious doubts about the role of JH in the control of alary polymorphism and the experiments reported here were carried out to determine the effect of crowding on the volume and ultrastructure of CA in apterous *M. viciae* in which alary polymorphism is controlled by a maternal physiological mechanism.

Isolated adult apterae, reared on ‘tic’ beans at 15±2 °C and a photoperiod of 16.5 h were prepared as alatae producers and apterae producers by confining them in specimen tubes either singly or in groups of 10 for 24 h (ref. 2). Aphids were fixed, embedded and stained for light and electron microscopy and the methods used are summarised in Table 1. Sagittal sections of whole aphids were cut serially and CA volume was estimated from planimetric measurements of consecutive sections in a series.

Table 1 Summary of methods used in fixing, embedding and staining *M. viciae* for light and electron microscopy

	Fixative	Embedding material	Section thickness	Stain
Whole aphid	Aq. Bouin + 0.5% TCA[19]	Celloidin and Paraplast	7 µm	Paraldehyde Fuchsin[19]
Heads	Formaldehyde-glutaraldehyde[20]	Araldite	0.5 µm 60–120 nm	Toluidine blue Uranyl acetate + lead citrate

The mean CA volume in apterae producers (22,804 µm^3; s.d. 5,660 µm^3; $n = 10$) is not significantly different from that of alatae producers (19,028 µm^3; s d. 4,888 µm^3; $n = 12$) ($P > 0.05$). Sagittal sections of the heads of three isolated and three crowded apterae were cut with glass knives. Thin sections were examined through an electron microscope (AEI model 6B) and the CA was identified with the aid of semithin sections (0.05 µm). Nuclei are irregularly shaped in both isolated and crowded apterae, they occur near the periphery of the gland, are often elongated and the nuclear membrane is highly invaginated. We therefore consider that nuclear dimensions cannot be used as a criterion of CA activity and that the contradictory descriptions of nuclear shape in active CA of other insect species[10–13] make it impossible to use shape as an index for JH synthesis and secretion in *M. viciae*. Although mitochondria are usually larger in active CA than in inactive CA[11,13], there is no significant difference between the mean sizes of fifty mitochondria from each of the six aphids examined ($P > 0.05$). Smooth endoplasmic reticulum, membranous whorls and cytoplasmic vesicules, which are characteristic of inactive CA in some insect species[14,15], occur in both isolated and crowded apterae, and the cytoplasm also contains numerous neurosecretory axons.

The results indicate that the synthetic and secretory activity in CA of apterae-producing and alatae-producing aphids are the same, since the volume, mitochondrial size and cytoplasmic membrane composition are similar in both groups of aphids. Nuclear dimensions cannot be used as an index of CA activity, since nuclei have an irregular shape in adult apterae, and changes in the ultrastructure of the endoplasmic reticulum (which is the best individual index of CA activity) occur as much within a larval, pupal or imaginal instar as they do between instars[14,15]. We therefore consider that differences in volumetric and nuclear dimensions between alatae producers and apterae producers and between alatiform and apteriform larvae can no longer be regarded as indicative of differences in CA activity[3,4,16]. We can find no evidence to support either White's[3] assertion that the maternal CA is involved in the prenatal control of alary polymorphism or Elliott's[16] suggestion that the CA is involved in the control of both ovarian growth and larval development in aphids. This does not mean that alary polymorphism is unaffected by JH concentration in the haemolymph since this may be controlled by the presence of specific metabolic enzymes[17] and protein carriers[18] which have been reported in other insect species.

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Manganese and photosynthetic oxygen evolution by algae

MANGANESE is required for the photoautotrophic growth of O_2-evolving organisms, in which it functions in the O_2-evolving complex of photosystem II. Mn-deficient cultures thus lose their O_2-evolving capacity, but activity can be restored by reincorporation of Mn into the photosynthetic apparatus. The ratio of Mn atoms to chlorophyll molecules in O_2-evolving organisms and preparations is 1:50–100 (ref. 1).

Heterocystous blue-green algae such as *Anabaena cylindrica* may provide a useful model for the study of the role of Mn in photosynthetic microorganisms since in cultures where all the cells are supplied with Mn, one cell type (the vegetative cell) evolves O_2, whereas the other (the heterocyst), which arises from vegetative cells by differentiation, does not[2]. We have therefore investigated the relationship between O_2 evolution and the Mn content of intact filaments and heterocysts of *A cylindrica*, to determine whether the inability of heterocysts to evolve O_2 could be explained on a biochemical/biophysical basis by a lack of Mn. We grew the algae in the presence of ^{54}Mn, measured ^{54}Mn incorporation and related this to the O_2-evolving capacity of various preparations.

Table 2 Mn–chlorophyll ratio, O_2-evolving capacity and photosystem I activity of *A. cylindrica* preparations

	Filaments	Heterocysts
Mn–chlorophyll ratio (g atoms:mol)	1:77	1:877
O_2 evolution (μmol per mg chl. per h)	97.3	0.0
Methylviologen supported photoreducing activity (μmol O_2 consumed per mg chl. per h)	—	252*
AT^{32}P formation (μmol ATP formed per mg chl. per h)	—	64*

O_2 evolution was measured in a reaction mixture of 2 ml containing 30 mM HEPES, *p*H 7.5, and 2 mM $K_3Fe(CN)_6$ as electron acceptor with the heterocysts. Methylviologen photoreduction was measured in a reaction mixture containing, in 3 ml of 30 mM HEPES buffer, *p*H 7.5: 2.5 mM NaN_3; 6.7 mM ascorbate; 5 × 10^{-5} M methylviologen, and 5 × 10^{-5} M dichlorophenol indophenol. AT^{32}P formation was measured in a reaction mixture containing, in 3 ml: 100 mM KCl; 6.7 mM ascorbate; *p*H 7.5, 30 mM HEPES; 6.7 mM phosphate containing 1–3 × 10^6 c.p.m. ^{32}P; 2 mM $MgCl_2$; 0.05 mM phenazine methosulphate; 3.3 mM ADP The ATP-trapping system contained hexokinase (Sigma type IV, 4 U) and 6 mM glucose Experiments were carried out at a light intensity of 7 × 10^5 erg cm^{-2} s^{-1} at 25 °C, and samples contained 15–40 μg chlorophyll. —,Not measured *Broken at 16,000 pound inch^{-2} before assay.

Before using *A. cylindrica,* we carried out experiments on two other organisms. *Chlorella vulgaris* was used to demonstrate the correlation between the Mn content and the O_2-evolving capacity of algal cells Table 1 shows that intact cells and lamellar fragments of *C. vulgaris* both evolve O_2, and in both cases the material is rich in Mn, with Mn–chlorophyll ratios of 1 : 26–33. The lamellar fragments, which have a somewhat lower capacity to evolve O_2 than the intact cells, have a slightly lower Mn content.

Intact spheroplasts of *Phormidium luridum*[3], evolve O_2 but when a Mn-containing low molecular weight 'Hill factor' is released from broken spheroplasts, their O_2-evolving capacity is lost. This latter capacity is restored when the factor is added back to the spheroplast preparations. Our data obtained with *P. luridum* (Table 1) provide quantitative support for the qualitative observation that the loss of the mn-containing factor results in O_2 loss They show that when O_2-evolving spheroplasts which have a Mn–chlorophyll ratio very similar to that of intact filaments are ruptured to release the 'Hill factor', the loss of O_2 evolution is accompanied by a decrease in the Mn–chlorophyll ratio of the lamellar fragments from 1:62 to 1 : 592. Such results provide evidence that the Mn is associated with the O_2-evolving reaction centre of photosystem II in blue-green algae.

In experiments with *A. cylindrica* (Cambridge Culture Collection No. 1403/2*a*) the alga was grown axenically in 2 l batch culture and ^{54}Mn (20 μCi) was added at the time of inoculation After 8 d, when the algae were in late log phase of growth, the cells were collected by centrifugation, washed three times in HEPES buffer (30 mM, *p*H 7.5) containing 1 mM EDTA, and finally resuspended in HEPES buffer Isolated heterocysts were prepared from such filaments by treatment with lysozome (1 mg ml^{-1}) in mannitol-HEPES buffer (0 5 M and 30 mM respectively, *p*H 7.5) for

Table 1 Mn content and O_2-evolving capacity of *C. vulgaris* and *P. luridum* preparations

Organism	Material	Mn–chlorophyll ratio (g atoms: mol)	O_2 evolution (μmol per mg chl.per h)
C. vulgaris	Intact cells	1:26	39
	Lamellar fragments	1:33	25
P. luridum	Intact filaments	1:51	107
	Spheroplasts	1:61	44
	Spheroplast fragments	1:592	4

C. vulgaris (Cambridge Collection No 211/11h) was grown in 2 l batch culture in the medium of Goulding and Merrett[6], at a light intensity of 4.5 × 10^3 erg cm^{-2} s^{-1} at 25 °C. The culture was bubbled with air+5% CO_2 (1.0 l min^{-1}). ^{54}Mn (10 μCi l^{-1}) was added at the time of inoculation of the alga and the cells were collected in late log phase. Cells were then washed in 30 mM HEPES (N-2-hydroxyethylpiperazine-N′2-ethanesulphonic acid) buffer, *p*H 7.5+1 mM EDTA, then suspended in HEPES buffer and broken by treatment (twice) in a Yeda press at 1,500 pounds inch^{-2}. Lamellar fragments remained in the supernatant after centrifugation of the broken cells at 300*g* for 5 min, and were collected by pelleting at 30,000*g* for 15 min. O_2 evolution was measured using a Rank-type O_2 electrode in 2 0 ml of 30 mM HEPES buffer containing 2 mM $K_3Fe(CN)_6$ *P. luridum* (Indiana University strain 426) was grown, preparations were obtained, and O_2 evolution was measured as before[3]. ^{54}Mn (10 μCi l^{-1}) was added at the time of inoculation and the material was collected in late log phase of growth. Chlorophyll was measured according to Mackinney[7]. Each value is the mean of duplicate determinations.

30 min at 37 °C. The pellet was resuspended in HEPES buffer, treated twice in a Yeda press at 1,500 pounds inch^{-2} under argon and diluted three to five times with HEPES buffer + 1 mM EDTA. Heterocysts were then separated from vegetative cell material by differential centrifugation in HEPES buffer. Samples of the intact filaments and of isolated heterocysts were then assayed for a capacity to evolve O_2, for photosystem I activity and for ^{54}Mn content. O_2 evolution was measured using a Rank-type O_2 electrode; photosystem I-dependent electron transport was measured as O_2 consumption by photoreduced methylviologen when ascorbate + DCPIP was supplied, and cyclic photophosphorylation was measured as AT^{32}P formation according to the method of Avron[4]. ^{54}Mn incorporation was measured as radioactive γ-emission using a well-type scintillation counter (Panax type SC-LP).

Table 2 shows that the inability of isolated intact heterocysts to evolve O_2, noted before[2], is accompanied by a depletion of Mn, the content of which in isolated heterocysts is only 9% of that in the O_2-evolving whole filaments. Such a lack of O_2 evolution is not accompanied by a general loss of photosynthetic functions by the heterocysts since they can carry out photosystem I-mediated electron transport and cyclic photophosphorylation, and have a normal chlorophyll content.

Our results thus provide evidence that not only is Mn associated with the photosystem II reaction centre of blue-green algae, but that heterocysts of N_2-fixing algae are deficient in Mn and a functional photosystem II. This sophisticated mechanism and possibly the lack of certain photosystem II pigments[5], ensures that in heterocysts there is no photosynthetically produced O_2 to inactivate the O_2-sensitive nitrogenase which occurs within these differentiated cells.

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Enzyme to break down lettuce endosperm cell wall during gibberellin- and light-induced germination

THE physiology of lettuce seed germination has been a subject of extensive study, but very little is known of the fundamental biochemical events which occur during germination. Here we report that the endosperm cell wall is largely composed of a mannose polymer (that is, a mannan); and that germinating seeds produce an enzyme capable of its breakdown.

The endosperm, a tissue two or three cell layers thick which completely surrounds the embryo, is a physical barrier which is ruptured by the growing radicle during normal lettuce seed germination It has been proposed by some that the endosperm is degraded by the production of a cellulase before radicle protrusion can take place[1]; others have maintained[2] that the germinating embryo itself develops sufficient thrust to penetrate the intact endosperm. It has been demonstrated by microscopy that substantial degradation, in an ordered fashion, of the endosperm cell wall does occur during germination[3]. Whether this is a necessary event for germination (radicle protrusion) to take place is still not clear; nor has the biochemical mechanism of this degradation been established until now. An enzyme produced in germinating seeds and capable of degrading the endosperm cell wall has been sought since Ikuma and Thimann reported some success in detecting a carboxymethylcellulase and a pectinase[1]; and it has been shown recently that there are indeed changes in extractable carboxymethylcellulase activity during germination[4].

One puzzling question arising out of the enzymic endosperm-degradation hypothesis is how the cell walls of the impinging radicle are themselves protected from degradation. Of the several possible explanations, perhaps the simplest is that there is some major difference in the composition of the radicle and the endosperm cell walls.

Accordingly, we have examined the total sugar composition of the cell walls of whole lettuce seeds, of dissected cotyledons, radicles and endosperm, and of 1-d-old seedlings (Table 1). This analysis shows that the endosperm, which contains two-thirds of the total seed cell wall polysaccharide material, has an unusual composition, comprising about 60% mannose. The cell walls of the cotyledons, radicles and the young seedling have considerably lower levels of mannose, and have a composition more typical of the cellulosic primary cell wall of higher plants[9]. The presence of mannose, initially identified by cochromatography with a mannose standard on gas–liquid chromatography, as the major sugar in the hydrolysate of endosperm cell walls was confirmed by both paper chromatography (ethyl acetate–pyridine–water, 8 : 2 : 1 v/v) and by the cysteine–sulphuric acid test[10]. The large amounts of mannose do not arise from a cytoplasmic storage polysaccharide (lettuce is not regarded as a carbohydrate-storing seed), since after homogenisation in ice-cold buffer, only 5% of the amount of polysaccharide precipitated at 3,000*g* is found in a 27,000*g* particulate fraction, where any storage bodies would be precipitated.

The occurrence of mannose in such a quantity in the endosperm cell wall means that a large portion of the cell wall polysaccharide is a mannan. This can be assumed to be a linear β-1,4 mannan (possibly a β-1,4 glucomannan, and perhaps with galactose side-chain substitution) on the basis of what is already known about other higher plant mannans[11]. The cell-wall mannan could be a component of the microfibrillar element shown to be present in the thick ramified lettuce endosperm walls[3]. Partially microfibrillar mannans also form a large part of the thickened cell walls of vegetable ivory nut (*Tagua*) endosperm[12]; and in the context of lettuce endosperm cell wall mechanical properties, the occurrence of cell-wall mannans in such a seed endosperm, noted for its hardness and mechanical rigidity is interesting.

The lettuce endosperm cell wall is broken down during germination, so the presence of an endogenous enzyme which can decompose the major cell wall component was investigated Table 2 presents the results of an experiment, incorporating a viscometric assay, which show that an enzyme activity occurs during germination which brings about a rapid degradation of locust bean (carob, *Ceratonia siliqua* L.) galactomannan, a polysaccharide which contains a β-1,4 mannan backbone chain[11]. This in indicative that germinating lettuce seeds produce an endo-β-mannanase (EC 3.2 1.78). The presence of an α-galactosidase, which can also bring about galactomannan viscosity changes at the expense of a considerable substrate hydrolysis[12], is unlikely, since incubation of the most active extract with galactomannan for 3 h led to a less than 1%

Table 1 Percentage of sugar composition of lettuce seed tissue cell walls (mg per 100 mg carbohydrate)

	Seeds imbibed in darkness for 3 h				Seeds imbibed in light for 23 h	
	Endosperm	Cotyledons	Radicle	Whole seeds	Endosperm	Seedlings (cotyledons and radicles)
Rhamnose	0.4	1.4	1.7	0.9	0.3	2.1
Fucose	0	0.6	0	0.3	0	1.1
Ribose*	0.5	2.9	5.2	2.3	0.7	3.7
Arabinose	9.5	18	15	16	11	17
Xylose	1.5	7.4	6.0	4.0	2.0	7.0
Mannose	58	4.8	7.4	39	53	3.7
Galactose	9.5	13	8.6	12	11	13
Glucose	10	18	28	13	12	23
Uronic Acids	10	34	28	12	10	29
mg Carbohydrate in hydrolysate per 50 seeds (~per 50 mg)	1.73	0.81	0.18	2.71	1.13	1.15

Batches of 50 lettuce seeds (*Lactuca sativa* var. Grand Rapids, Ferry Morse Seed Co. 1971) were imbibed for 3 h, or germinated in white light for 23 h. Embryos plus endosperm were separated from the surrounding layers and then dissected out under a dissecting microscope. The tissues were placed immediately in 70% ethanol and ground in a Micro-Dounce Homogenizer. The cell-wall material was collected by centrifugation at 3,000*g* for 10 min, and washed once with alcohol and twice with chloroform–methanol (2:1 v/v) then dried by rotary evaporation. This wall preparation was dissolved in 0.05 ml 72% w/w sulphuric acid for 3 h at room temperature, then diluted to 4% w/v with 1.4 ml water and hydrolysed in a sealed tube for 1 h at 120 °C. The hydrolysate was neutralised with barium carbonate and made up to 4.0 ml. Aliquots were assayed for total sugar[5] and acidic sugars[6], and analysed for neutral sugar composition by gas–liquid chromatography of the derivatised alditol acetates[7], on a 2.7 m×2 mm glass column (0.2% polyethyleneglycoladipate, 0.2% polyethyleneglycolsuccinate, 0.4% XF-1150 (ref. 8) on Chromosorb W, H.P., 80–100 mesh, (Chromatographic Specialties Ltd)) at 182 °C using helium carrier gas (30 ml min^{-1}) in a Hewlett–Packard 402 Gas Chromatograph. Neutral sugar composition was calculated from measured peak areas using correction factors previously established with sugar standards; total sugar composition was computed using determined neutral sugar composition and known different behaviour of individual standard sugars in each of the two assays.

*Presumably present as cytoplasmic contamination.

release of potential reducing power (assayed by the ferricyanide method[14]).

Endo-β-mannanase enzyme activity was seen both in light- and gibberellin-induced germination. Activity seems to be correlated with the degree of germination, as measured by radicle protrusion. Minimal activity was detected when the germination index was low (10 h) and none was found in seeds imbibed for only 1 h. It is not possible to say yet whether enzyme production precedes or is necessary for radicle protrusion.

Endo-β-mannanases are known to be involved in the degradation of storage galactomannans in germinating legume seeds[12]. Although the report elsewhere of carboxymethylcellulase activity detectable during the same early period in germinating lettuce seeds suggests that the endo-β-mannanase we have identified may have poor substrate specificity[4]; that "carboxymethylcellulase" has little or no available cellulose substrate in the endosperm cell wall is quite clear. If a cellulase is indeed produced in germinating lettuce seeds, it cannot by itself lead to appreciable degradation of the endosperm cell wall; it might contribute, or may have some other, as yet unclear, function Since the composition of the endosperm cell wall does not change significantly over the first day of germination, while the total amount of cell wall polysaccharide present is decreased (Table 1) and the endosperm tissue becomes much easier to homogenise, this suggests that other cell-wall degrading enzymes are present. These observations are consistent with the endosperm cell wall degradation reported by Jones[3]. Unfortunately, Jones did not specify whether degradation takes place over the entire endosperm, or is localised to one region, such as, for example, the micropylar end of the seed from which the germinating radicle emerges; this is a very important detail.

Such questions as when and where in the seed the

Table 2 Galactomannan breakdown by lettuce seed extracts

Treatment	Time after imbibition (h)	Units enzyme activity per 1 g seeds	% Germination
Darkness	1	0	0
	10	0	0
	13.5	2.6	1±1
15 min red light after 2 h imbibition	10	4.8	1±1
	13.5	25	77±3
100 μg ml^{-1} GA$_3$ in darkness	10	0	
	13.5	9.6	17±3

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Batches (1g) of Grand Rapids lettuce seeds were incubated as shown above, ((9-cm Petri dish on two layers Whatman No.1 filter paper with 6 ml liquid at 23 °C), collected and ground in a total of 8 ml ice-cold McIlvaine's buffer, *p*H 5.0, and centrifuged at 17,000*g* for 10 min. This supernatant was fractionated by taking a 20–60% saturated ammonium sulphate cut[13], resuspending in half-strength buffer, and dialysing overnight at 4 °C against tenth-strength buffer. After a final clearing centrifugation at 17,000*g* for 10 min, the extract was made up to 4.0 ml with tenth-strength buffer. Locust bean galactomannan (Sigma) was purified by dissolving in water and precipitation from solution with 80% ethanol (twice), redissolved and freeze dried (analysis: 70% mannose, 28.5% galactose, 1% ribose). Decrease in viscosity was measured in a Cannon–Ubbelohde suspended level viscometer at 30 °C. The assay mixture consisted of 10 ml 0.1% galactomannan (dissolved in tenth-strength buffer) and 1 ml of the lettuce seed extract. The rate of decline of viscosity in active fractions was linear over the first hour. The enzymic activity per 1 g seeds is shown, as well as the degree of germination (radicle protrusion) at the time of collection. A unit of activity is defined as that which will bring about a 1% decrease in viscosity in 30 min.

3-min boiled extract showed no activity.

endo-β-mannanase is produced, and how this process is controlled, are of interest in the study both of lettuce seed germination and of the control of enzyme production in plants.

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Reduced sperm motility in the isthmus of the rabbit oviduct

SPERM transport through the female reproductive tract has been studied extensively in mammals[1,2], but no systematic evaluation of the motility of spermatozoa at different levels of the tract after coitus has been reported. We describe a procedure for collecting oviducal spermatozoa and present observations on an unexpected reduction in the proportion of motile spermatozoa in the isthmus of the rabbit oviduct over the 12-h interval between mating and fertilisation.

Thirty-six New Zealand White does were each mated with three or four bucks. Six does were killed at 1.5, 4, 6, 8, 10 and 12-h post coitus by intravenous overdose of sodium pentabarbitone (Nembutal, Abbott). Haemostats were clamped across the utero–tubal and isthmo–ampullary junctions of the oviduct and across the uterine horns above the cervices. The uteri were removed by cutting across haemostats placed below those clamping the utero–tubal junctions. Uterine sperm were collected by aspiration, after injecting 0.5 ml of a 5% mixture of rabbit serum (previously stored at −20 °C) in Tyrode's solution (*p*H 7.4) into each horn. To collect oviducal sperm, isthmic and ampullary segments of the oviduct were cannulated at their ovarian ends with 10 λ micropipettes (Yankee Disposable Micropipette, Clay Adams) introduced 1–2 mm into the lumen. Oviducal contents were flushed into the micropipettes by introducing the blunted needle of a Butterfly-23 Infusion Set (Abbott) 2–5 mm within each segment and injecting 10–20 λ of serum–Tyrode's. The tubing of the infusion set was mounted in a micropipette controller (Clinac Micropipetter, Lapine), which was used to regulate the volume and rate of fluid delivery.

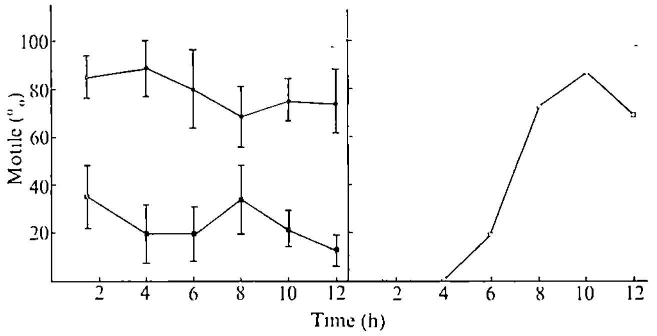

Fig. 1 Mean % of motile rabbit sperm ±s d. recovered from uterine horns (●) and Fallopian tube isthmi (■) during 12 h post coitus; data based on sperm counts in samples from 12 uterine horns and tubal isthmi at each interval. The proportions of motile ampullar sperm (□) are calculated from the sum of motile and non-motile sperm recovered from 12 ampullae at each interval.

Uterine and oviducal contents were examined immediately at room temperature by phase-contrast microscopy and sperm showing any evidence of flagellar movement were classified as motile: those lacking any activity were considered non-viable. All sperm were examined for the state of the membranes overlying the acrosomal region of the head (Table 1). The proportion of motile sperm in homogeneous uterine samples was estimated by counting 100–200 sperm from each horn; all sperm in ampullar samples were counted. Motile isthmic sperm flushed from 10-λ micropipettes were not distributed randomly on microscope slides, so they were counted in sets of 100 until the proportion of motile cells in successive sets varied by less than 5% from the percentage motile sperm in the cumulative total.

To exclude the possibility that estimates of isthmic sperm motility were biased by differential recovery of non-motile sperm, the contents of ten isthmi were collected in three serial flushes. The first flush of 10 λ was collected as described. The second was 0.5 ml of serum–Tyrode's, followed by 0.5 ml of 1% sodium dodecyl sulphate (SDS) in distilled water. These were collected directly in 0.5 ml polyethylene centrifuge tubes and sperm were concentrated by centrifugation for 30 s in a Beckman microcentrifuge (Model 152). Two isthmi at 4, 8 and 12 h post coitus and four isthmi at 6 h post coitus were flushed in this way. The mean percentage of motile spermatozoa in 10-λ and 0.5 ml serum–Tyrode's samples did not differ significantly (22.1%±15.2 as against 17.0%±10.0; $P>0.25$, $f=0.0073$ (1,18) by one-way analysis of variance). The number of sperm recovered in 0.5 ml SDS flushes was less

Table 1 Motility and state of the head membranes of sperm recovered from the reproductive tract of female rabbits between mating and fertilisation

	Uterus			Isthmus			Ampulla		
Hours post coitus	No. counted	Motile* (%)	Non-motile† altered heads	No. counted	Motile* (%)	Non-motile† altered heads	No. counted	No. motile‡	No. non-motile altered heads
1.5	900	85.0±8.8	7.8±5.1	287	35.1±13.0	36 0±16.8	5	0	4
4	1,200	88.8±11.6	5.6±7.4	3,858	19.0±12.1	55.9±16.3	6	0	4
6	1,200	80 7±15.9	11 6±11.7	5,689	19.6±10.5	68.2±14.5	21	4	10
8	1,300	68.1±13.2	12.2±8.8	6,064	33.5±14.3	50.2±13.9	60	43	9
10	1,500	75.6±8.6	10.5±6.6	5,118	21.8±6.9	48.6±10.0	166	145	15
12	1,600	74.7±13 0	13.9±9.3	6,690	12.8±6.1	66.3±15.5	139	97	19

*Mean %±s d. of intact motile sperm recovered from 12 uterine horns and 12 Fallopian tube isthmi at each post coital interval.
†Mean %±s d. of non-motile sperm with lifted or absent acrosomal head membranes The remainder of non-motile sperm recovered in uterine and isthmic samples either seemed to have intact head membranes, with phase-dense acrosomes, or were aggregated with leukocytes.
‡Ampullar sperm numbers are based on the total number of sperm recovered from all of 12 ampullae sampled at each post coital interval.

than 1% of the 2,000–4,000 previously estimated to be present in the isthmus at these times[3–7]. These data support our assumption that the proportion of motile sperm in 10-λ samples is a valid representation of the motility of the total population recoverable from the isthmus in the first 12 h post coitus.

Seventy per cent or more, of uterine spermatozoa were motile at all collection times and most showed vigorous progressive movement (Table 1, Fig. 1). In contrast, the motility of isthmic sperm was uniformly poor, with few showing progressive movement and usually less than 35% any sign of flagellar activity. Motile ampullar sperm invariably showed vigorous motility of better quality than that of isthmic sperm from the same oviduct, and their motility often exceeded that of uterine sperm.

Most non-motile sperm throughout the female tract exhibited morphological changes in the membranes overlying the sperm head (Fig. 2). These changes varied from a lifting of the acrosomal cap to complete loss of the acrosome. Non-motile sperm with altered head membranes usually constituted less than 15% of all uterine and ampullar sperm, compared with 50%, or more, from the isthmus (Table 1). Motile sperm with altered or missing acrosomal caps were not observed in uterine samples and were seen only rarely in isthmic samples. In comparison, motile ampullar sperm with lifted or missing acrosomal caps were seen frequently at 8 h post coitus and thereafter.

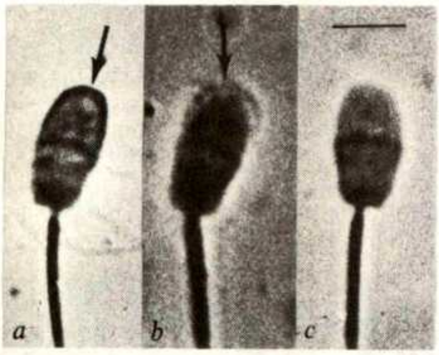

Fig. 2 Rabbit sperm collected 10 h post coitus from the isthmus of the Fallopian tube; phase-contrast microscopy. *a*, Sperm with intact, phase-dense acrosome (arrow at acrosome margin); *b*, non-motile sperm with altered head membranes (arrow on edge of expanded acrosomal cap); *c*, non-motile sperm with absent acrosomal cap. Calibration bar = 5 μm; × 1,900.

These findings are unexpected in the light of literature on sperm transport[1,2] and capacitation[8,9] in mammals, since it has been generally assumed that spermatozoa present throughout the oviduct before ovulation would be highly motile. The reduction in number of viable sperm in the isthmus seems to be a means of regulating sperm transport not previously appreciated. Together with the cervix and utero–tubal junction, the isthmus has been considered to restrict the passage of sperm to the site of fertilisation[10,11], but a physiological basis for its action was unknown. Whether the isthmus solely reduces the number of sperm entering the ampulla, to protect against polyspermic fertilisation[10,11], or whether removal of defective or incompetent sperm also occurs in this region[12] remains to be determined. Our finding that a high proportion of non-motile isthmic spermatozoa have disrupted or missing acrosomes, whereas similarly altered ampullar sperm are often motile, suggests that: (1) the adverse influence of the isthmic environment may be directed against the plasma membrane of the sperm head, and (2) spermatozoa remain viable in the isthmus by virtue of stable plasma membrane configurations. Such sperm may be considered to be a selected population. The virtual absence from the isthmus of motile spermatozoa with altered head membranes indicates that the stimulus for induction of specific acrosomal changes which precede fertilisation may be limited to the upper oviduct.

The reduced motility and altered morphology of isthmic sperm may depict their condition *in vivo*, or may be manifestations during recovery of other physiological changes undergone within the oviduct. Previous investigations of sperm transport in the female rabbit focused on the number of spermatozoa present in various regions of the tract[3–7] and often used spermicidal detergents to aid recovery. Moreover, observations on the physiology of spermatozoa in the oviduct have usually involved direct transfer of uterine sperm to the ampulla, thus bypassing the utero–tubal junction and tubal isthmus. It is not surprising, therefore, that the phenomenon of reduced sperm viability in the isthmus and its implications for understanding sperm transport and capacitation in the female rabbit have gone unrecognised.

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Nuclear transplantation in the rabbit egg

SINCE the experiments of Briggs and King[1], in which nuclei from blastula cells were transplanted by microinjection into enucleated frogs' eggs, the technique of nuclear transplantation has been used extensively to study interactions between nucleus and cytoplasm during differentiation in amphibians[2–5]. But severe difficulties are encountered in attempts to apply this technique to the mammalian egg. The rabbit egg, with a diameter of 100 μm, is 1,000 times smaller in volume than a frog's egg, and the mouse egg has a volume one-third that of a rabbit's egg. An alternative, and technically easier method of transplanting a nucleus is by inducing fusion of an egg and a cell with Sendai virus. The technique of virus-induced fusion, originally developed for hybridising somatic cells[6,7], has been adapted to fuse somatic cells with unfertilised or fertilised mouse eggs[8–13]. Although Sendai virus was shown not to harm mouse embryos in these experiments, and cells were successfully fused, the transplanted nuclei did not survive. Unfertilised mouse eggs which were fused with cells from embryos at the 2–8-cell stage failed to cleave[8].

I report here experiments in which nuclei of embryonic cells were introduced into unfertilised rabbit eggs by both microinjection and virus-induced fusion. Unfertilised eggs were recovered from the Fallopian tubes of females induced to ovulate by a single intravenous injection of chorionic gonadotrophin (Chorulon, Intervet). In virus-induced fusion experiments which required denuded eggs the zona pellucida was removed mechanically.

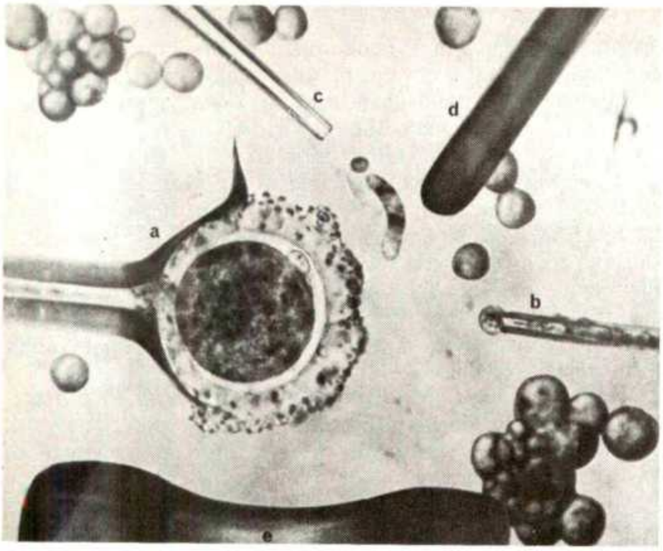

Fig. 1 General arrangement of instruments, egg and donor cells (×300). *a*, Cup, holding egg; the spike projecting from the cup is for removing sticky cytoplasm and corona cells from the tip of the injection micropipette. A single polar body is visible in the perivitelline space of the egg. *b*, Injection micropipette, containing a donor cell. *c*, 'Sausaging' pipette for deforming a donor cell into a cylinder before it is taken into the injection micropipette. *d*, A flame-polished needle for manipulating eggs and cells. *e*, Large bore take-up pipette for removing eggs after microinjection. During an experiment instruments, eggs and donor cells are in several focal planes; in this photograph they have been arranged in a single plane of focus for clarity of presentation.

Donor cells were obtained from morulae recovered from females mated 48 h previously. In the first series of experiments the donor nucleus was introduced into the egg at any stage of its cycle. In the second series the aim was to synchronise donor and egg. When ovulated, the unfertilised rabbit egg, in common with most mammalian eggs, is arrested at metaphase II, awaiting the stimulus to complete meiosis which is normally provided by sperm penetration. A nitrous oxide (N_2O) block is effective in arresting somatic cells in mitosis, and the effect is reversible[14–16]. This technique was therefore applied to rabbit morulae in these experiments to increase the proportion of nuclei in mitosis in the donor cell population. In 54 morulae (1,191 nuclei) incubated for 4 h in an atmosphere containing N_2O at a pressure of 5 kg cm^{-2}, 202 nuclei (16.9%) were in mitosis, three times as many as in an untreated population.

To identify the donor nucleus, or its cleavage products, after transfer to the egg, tritiated thymidine (^{3}H-TdR; specific activity 12.6 Ci mmol^{-1}, Radiochemical Centre) was added to the culture medium of the donor embryos to give a final concentration of 0.25 μCi ^{3}H-TdR ml^{-1}. After 4 h of incubation, 91.6% of non-synchronised, and 87.8% of 'synchronised' nuclei were labelled.

To obtain single cells for use in nuclear transfer experiments the zona pellucida was cracked, the morula cells were released and where necessary cells in clumps were separated by bouncing them apart on an agar mattress.

In all operations to transplant nuclei into eggs, whether by microinjection or by virus-induced fusion, the eggs and cells were manipulated in the well of a glass chamber on a microscope stage at magnification of ×200–×400. The

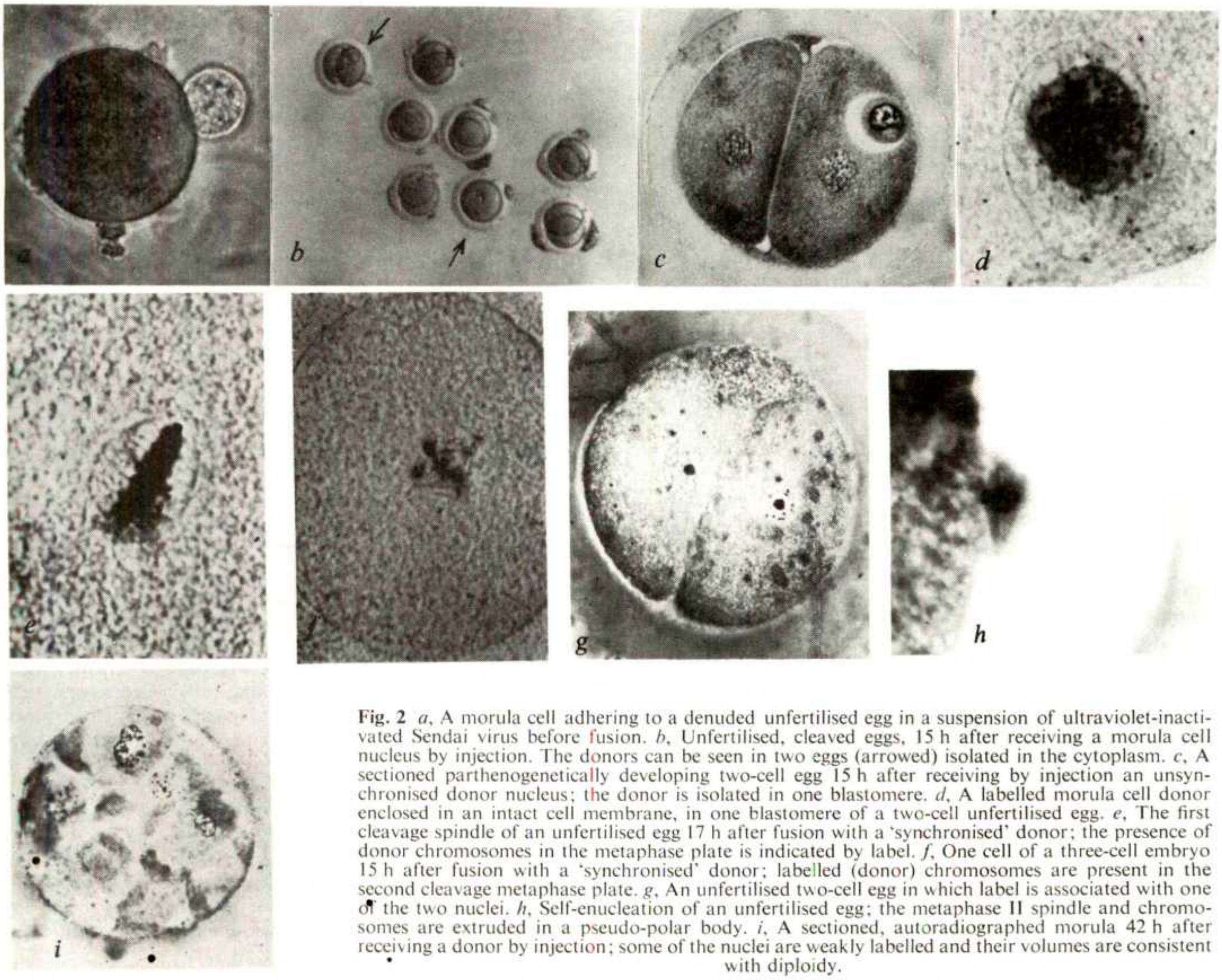

Fig. 2 *a*, A morula cell adhering to a denuded unfertilised egg in a suspension of ultraviolet-inactivated Sendai virus before fusion. *b*, Unfertilised, cleaved eggs, 15 h after receiving a morula cell nucleus by injection. The donors can be seen in two eggs (arrowed) isolated in the cytoplasm. *c*, A sectioned parthenogenetically developing two-cell egg 15 h after receiving by injection an unsynchronised donor nucleus; the donor is isolated in one blastomere. *d*, A labelled morula cell donor enclosed in an intact cell membrane, in one blastomere of a two-cell unfertilised egg. *e*, The first cleavage spindle of an unfertilised egg 17 h after fusion with a 'synchronised' donor; the presence of donor chromosomes in the metaphase plate is indicated by label. *f*, One cell of a three-cell embryo 15 h after fusion with a 'synchronised' donor; labelled (donor) chromosomes are present in the second cleavage metaphase plate. *g*, An unfertilised two-cell egg in which label is associated with one of the two nuclei. *h*, Self-enucleation of an unfertilised egg; the metaphase II spindle and chromosomes are extruded in a pseudo-polar body. *i*, A sectioned, autoradiographed morula 42 h after receiving a donor by injection; some of the nuclei are weakly labelled and their volumes are consistent with diploidy.

Table 1 Introduction of a morula cell nucleus into an unfertilised rabbit egg by microinjection

	No. of eggs injected	Eggs surviving* injection procedure		No. of living eggs surviving incubation for up to 42 h	No. of eggs containing a donor nucleus			
		No.	%		1 Cell	Cleaved	Total	% of eggs injected
Donors not synchronised	528	372	70	271	15	12	27	5.1
Donors 'synchronised'	65	56	86	39	3		3	4.5
Total	593	428	72	310	18	12	30	5.0

*Survival assessed immediately after injection.

chamber was cooled to 5 °C to administer a cold shock to the eggs as a stimulus to activation and to assist adsorption of the virus.

Eggs for injection were held in a cup, a donor cell was taken into a pointed micropipette of 8–10 μm inner diameter and injected into the egg (Fig. 1). In virus-induced fusion experiments the denuded eggs and donor cells were fused by ultraviolet-inactivated Sendai virus at a concentration of 300–400 haemagglutinating units of virus per ml. After exposure of denuded eggs and donor cells separately to the virus suspension for 10 min each egg was rolled on to a cell, which then adhered to the vitelline membrane before fusion (Fig. 2*a*).

In only 30 of 593 eggs (5%) which received a morula cell nucleus by injection could the donor be identified positively in the egg (Table 1). A higher proportion (18.8%) of nuclei were identified in eggs after introduction by virus-induced fusion (Table 2).

nucleus having been lost by self-enucleation. Of 155 injected eggs examined after nuclear transfer and culture for 13–27 h, 7.1% did not contain an egg nucleus, and in a further 5.2% the egg chromosomes were dispersed in the cytoplasm and so unlikely to take part in development. Of the anucleate eggs, 63.6% (4.5% of all injected eggs) had extruded the egg metaphase II chromosomes in a pseudo-polar body (Fig. 2*h*). Evidence suggesting that nuclear transplantation into enucleated eggs has been achieved is provided by four morulae cultured for 42 h after the injection of a labelled donor. These morulae had cleaved regularly to 18–26 cells, at a rate similar to that of the fertilised egg *in vivo*. Label at a low level was associated with 40 of 86 nuclei (46.5%), and the nuclear volumes were consistent with diploidy (Fig. 2*i*).

Clearly a somatic cell nucleus, transplanted into an unfertilised rabbit egg, can replace the sperm in supporting development in the early cleavage stages. After 'fertilisation' of the egg by the transplanted nucleus the synkaryon or 'zygote' is capable of apparently normal division at least up to the morula stage. Although such development is rare, the fact that it is possible extends to the rabbit, and by inference to other mammals, the possibility of experiments which have so far been restricted to amphibians. Conclusive proof of the development of an embryo from a somatic cell nucleus transplanted into an enucleated rabbit egg has not been demonstrated; however, there is no apparent reason why a donor nucleus which can fuse with the egg nucleus after transplantation should not also be able to support development by taking the place of the egg nucleus in an anucleate egg.

Table 2 Introduction of a morula cell nucleus into an unfertilised rabbit egg by virus-induced fusion

	No. of eggs	Eggs surviving* fusion procedure		No. of living eggs surviving incubation for up to 27 h	No. of eggs containing a donor nucleus			
		No.	%		1 Cell	Cleaved	Total	% of eggs fused
Donors not synchronised	54	38	70	20	7		7	13
Donors 'synchronised'	47	47	100	38	9	3	12	25.5
Total	101	85	84	58	16	3	19	18.8

*Survival assessed immediately after removal from virus suspension.

After nuclear transfer either by microinjection or by virus-induced fusion, in the best cases the recipient eggs were activated and cleaved regularly, at a rate similar to that of the fertilised egg *in vivo*. In eggs containing both host and transplanted nuclei the egg nucleus usually developed independently of the donor but in some cases the two nuclei fused. Donor nuclei which did not fuse with the egg nuclei were found in one blastomere of eggs that had cleaved (Fig. 2*c*), where they either became pycnotic, or remained unchanged where isolated from the egg cytoplasm by an intact cell membrane (Fig. 2*d*). When nuclei fused, a single spindle and metaphase plate formed at the centre of the egg on which both egg (unlabelled) and donor (labelled) chromosomes assembled (Fig. 2*e*).

Evidence of fusion of egg and donor nuclei has been obtained only in experiments in which 'synchronised' donors have been transplanted into the eggs. An interpretation, consistent with evidence from other studies concerned with somatic cell hybridisation[17], is that for the donor nucleus to combine with the egg nucleus, and then to take part in apparently normal division, both nuclei must be in synchrony. The evidence indicates that nuclei in or near division, that is late G_2, M or G_1, may become synchronised with the egg, whereas nuclei in S phase are asynchronous and do not take part in normal development. It may be supposed that the degree of synchrony necessary to permit fusion of donor and egg nuclei in a normal unfertilised egg is also required for the successful substitution of donor for host nucleus in an egg that has been enucleated.

In some cases the first cleavage metaphase plate may have comprised only labelled (donor) chromosomes, the egg

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Motility, diffusion and cell concentration affect pair formation in *Escherichia coli*

AT the high cell densities normally used in *E.coli* matings (about 10^8 cells per ml) individual cells undergo frequent and repeated collisions, and should therefore have multiple opportunities for pairing[1–3]. A mating pair of cells, once formed, will similarly continue to collide almost as frequently with other cells. It is not known whether these secondary collisions affect either the speed or extent of DNA transfer, since the true properties of a mating pair of cells formed with little likelihood of further collisions have not been studied. Pairs formed at low cell concentrations, however, should be suitable for such studies; indeed, they could be regarded as paradigms of simple cell–cell interactions. We show here that at low densities pair formation is much more efficient than at high densities, and also that motility decreases this efficiency once cells have collided.

It was necessary to estimate both the number of collisions between cells and the number of pairs formed. Cell movement was observed directly and the formation of progeny was used as an index of pairs formed. Since shaking introduces additional relative cell movement[4], all matings were carried out without shaking.

In the low density matings, inputs of both donor and recipient cells of about 10^5 per ml gave well defined progeny counts and showed exponential growth of both parents during 1 h. The selected marker, *purE*$^+$, enters the recipient cell approximately 4 min after DNA transfer starts[5]. The formation of progeny and the viable counts during such an experiment using non-motile strains are shown in Fig. 1. The rate of formation of progeny accelerates steadily, and their number does not exceed 0.1% of either parent during the experiment. The lag in the appearance of progeny strongly suggests that an unstable pairing which is sensitive to the experimental handling conditions precedes the more stable pairing which survives the pipetting and mild vortexing used. This suggests the model

$$\mathrm{D+R} \rightleftharpoons \mathrm{D\text{–}R_{unstable}} \longrightarrow \mathrm{D\text{–}R_{stable}}$$

where D represents donors, R recipients and D–R$_{\mathrm{stable}}$ the complexes which give rise to progeny colonies. For practical purposes, however, we have estimated a minimum value for the rate constant from the simplified model

$$\mathrm{D+R} \xrightarrow{k} \mathrm{D\text{–}R_{stable}}$$

assuming a time lag for the conversion of any pair into a stable pair of about 9 min, as the data suggest.

The experimental data from low density matings could be fitted to the simplified model at early times but the numbers of progeny found during the later stages of the experiment consistently exceeded expectation. We have observed under the microscope that both cells in apparent mating pairs, when placed on agar blocks and incubated further, continue to divide. We have therefore assumed that the number of progeny cells (P) can increase due to multiplication at a rate characteristic of the recipient strain. The equations used to model the process were

$$\begin{aligned} \mathrm{d}P/\mathrm{d}T &= kDR + k_R P \\ \mathrm{d}D/\mathrm{d}T &= k_D D \\ \mathrm{d}R/\mathrm{d}T &= k_R R \end{aligned}$$

where k_D and k_R are the observed growth rate constants for donor and recipient respectively. The theoretical numbers of progeny were computed using the cell numbers at the time of mixing as the initial conditions. The value of the second order rate constant, k, used to generate the theoretical curve (which incorporates the lag previously mentioned) shown in Fig. 1 was 7×10^8 l per 6×10^{23} cells per s. These units facilitate comparison of the rate constant with the upper limit for a second order rate constant in liquids for a reaction between neutral molecules, 3×10^9 l mol^{-1} s^{-1}, in a diffusion-limited reaction[9]. Pairing is therefore an inherently efficient process following the initial contact, as suggested by Nelson[10], particularly since not all cells in the population may be competent to mate[4,10] and some mating pairs will fail to produce recombinants for the selected marker[11].

The apparent rate constant for the mating (at low density) between motile parental strains is, as expected, significantly greater than for non-motile strains (Table 1). The apparent rate constants for the two crosses involving one motile and one non-motile parent, which might be expected to be similar, show an interesting asymmetry; the cross between a motile donor strain and a non-motile recipient strain is more fertile than the complementary cross. But the significance of the difference between the first two crosses mentioned, and of the asymmetry in the latter two crosses, can be evaluated only if the contribution of cell movement to the collision rate is known.

To this end, we included in these experiments measurements of cell movement. During each mating, videotape records were made of microscopic fields of samples from the parental cultures, observed in a closed chamber at 37 °C, while the bulk cultures were mated. The recordings were replayed to obtain

Table 1 Effect of motility and concentration on the relative efficiency of mating pair formation

Mean parental cell numbers per ml	Motility of strains		Relative mutual diffusion coefficients	Observed rate constant $\times10^{-10}$ (l per 6×10^{23} cells per s)	Relative efficiency of pair formation
	Donor	Recipient			
1.6×10^5	−	−	1	0.07	1
	+	+	19.8	0.6	0.43
	+	−	9.3	0.6	0.92
	−	+	11.4	0.09	0.11
3.2×10^6	−	−	1	0.1	1.43
	+	+	48.8	1.3	0.38
3.6×10^8	−	−	0.8	0.006	0.11
	+	+	4.0	0.0015	0.0054

Three sets of matings were carried out; within each set there was an approximate 1:1 ratio of donor to recipient cells, and a similar total cell input. The donor strains were B8 (motile) and ED1064 (non-motile); recipient strains were χ478 (motile) and ED1065 (non-motile). The values of the relative mutual diffusion coefficients and of the relative efficiencies of pair formation were chosen to be 1 for the cross between non-motile parental strains at the lowest concentration.

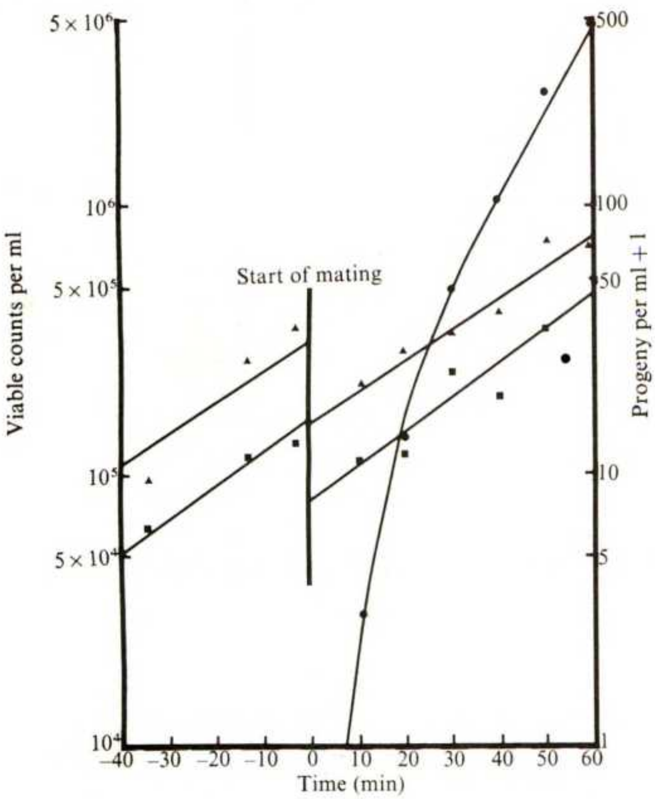

Fig. 1 Progress of the mating between the *E. coli* strains ED1064 and ED1065. These were isolated as spontaneously arising non-motile derivatives from, respectively, cells of Hfr B8 (refs 5 and 6; *metB*) and χ478 (ref. 7; F⁻*proC purE trp lys metE leu lac str*) that had survived a challenge from bacteriophage χ (provided by E. Meynell), which only attacks motile cells[8]. Cultures were grown in L broth at 37 °C to about 2×10^8 cells per ml, diluted to about 10^4 cells per ml in fresh L broth and grown for a further 90 min to approximately 2×10^5 cells per ml in a rotary shaking water bath (100 r.p.m.). Equal volumes of donor and recipient cultures were then mixed; incubation continued without shaking. Separate samples were diluted as appropriate in buffer, vortexed gently and pipetted into molten agar held at 46 °C; these samples were then poured on to different supplemented minimal agars selective for Pur^+ (Str^r) progeny and also for each parental strain. Viable counts per ml of donor (▲) and recipient (■) and the number of progeny per ml + 1 (●) are shown semi-logarithmically with the curve of the predicted progeny per ml + 1, assuming that $k = 7 \times 10^8$ l per 6×10^{23} cells per s.

tracings of the tracks of individual cells for the calculation of apparent diffusion coefficients. The relative magnitudes of the upper limits for the rate constants are directly proportional to the sum of the apparent diffusion coefficients for donor and recipients; we assume that the cell–cell distance on contact is the same in all crosses. From the ratio of the observed rate constants to the relative upper limits in these matings, we can calculate the relative efficiencies of pair formation per collision (Table 1).

The most efficient matings are those between the two non-motile strains at the lower densities. The corresponding crosses between the motile strains B8 and χ478 are about one-third as efficient. Since the average interval between successive collisions involving a given cell at the lowest concentration exceeds the duration of the experiment, this lower efficiency is an inherent property of mating pairs of motile cells.

The relative efficiencies of the matings involving one motile and one non-motile parent show that the asymmetry noted above is not due to different mobilities of strains B8 and χ478. Therefore other possibilities, including vectorial movement by the donor strain, must be considered.

Cell concentration also affected the relative efficiency. Thus, when one allows for a tenfold drop in the diffusion coefficient of the motile cells (but not of the non-motile cells) at the highest concentration, the crosses between motile cells and between non-motile cells were both ten times less efficient. These low efficiencies may be due to the additional collisions, which could result in disruption of existing pairs or the addition of further cells to existing complexes. We therefore suggest that physiological and genetic analysis of low and moderate density matings will be the most reliable source of information about the behaviour and properties of a simple mating pair of cells.

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Migration and chemotaxis of anucleate cytoplasmic leukocyte fragments

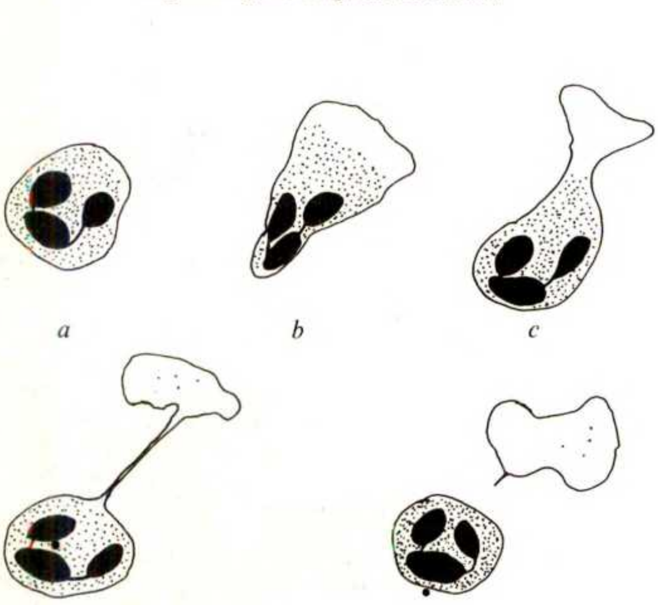
KNOWLEDGE of the mechanisms involved in leukocyte locomotion is limited. Although it is known that amoebae require a nucleus for locomotion[1,2] no similar studies have been done with leukocytes. Nothing precise is known about the cellular factors involved in the perception of a chemotactic gradient and the translation of this information into directional migration. We have investigated this problem using anucleate cytoplasmic fragments and heat-polarised granulocytes.

When leukocytes were heated to 46 °C on a microscopic stage under a coverslip, pronounced pseudopods were produced, resulting in anucleated cytoplasmic fragments. The stages of this process are summarised schematically in Fig. 1. Heating resulted in the gradual polarisation of the cell into two parts (Fig. 1*a–d*), one containing the nucleus and most of the phase-dense granules, the other consisting mainly of the hyaloplasm, containing few or no phase-dense granules. When polarisation occurred the cytoplasmic part moved away from the less mobile nucleated section. This process was reversible if heating stopped. Continued heating, however, resulted in the irreversible formation of anucleated cytoplasmic fragments (Fig. 1*e*). The behaviour of cells and fragments was analysed by microcinematography and video recording and, subsequently, the cell path was traced.

We consistently observed random migration of viable cytoplasmic fragments, while the nucleated part showed little or no motility. Previous studies had shown that neutrophils respond chemotactically to red blood cells which have been destroyed by a laser microbeam (necrotaxis)[4]. We found that viable cytoplasmic fragments are also capable of directional migration, resulting in the accumulation of fragments around the chemotactic target. We have demonstrated that the fragments can ingest bacteria[3]. Thus in contrast to amoebae[1,2], granulocytes do not require a nucleus for locomotion and chemotaxis, at least not for a limited time. Furthermore, only part of the cell membrane is needed for recognition of a chemotactic gradient and for phagocytosis. This conclusion agrees with observations[5] showing a segmental response of macrophages to phagocytic stimuli. It is interesting to compare the functional capacity of cytoplasmic fragments from leukocytes with the behaviour of blood platelets, which also represent cytoplasmic fragments. Even though it is well established that platelets contain contractile elements[6], we could not detect locomotion or chemotaxis. The structural basis for the differences between leukocyte fragments and platelets remains to be studied.

We have analysed the behaviour of heat-polarised leukocytes in order to obtain further information about the mechanism of the chemotactic response. In the absence of chemotactic stimulation, heat-induced pseudopod formation occurs randomly in all directions. Chemotactic stimulation considerably enhances heat-induced pseudopod formation, particularly in the early phases (Fig. 1*b* and *c*). More than 90% of the pseudopods extended and moved towards the chemotactic target, while the nucleated part either remained in its original position or slowly followed the cytoplasmic part. It has been suggested that leukocytes recognise a chemotactic gradient by means of receptors on the cell surface[7,8] and that pseudopod formation, leading to directional migration, takes place at the site of the membrane, which undergoes the most intense chemotactic stimulation[9]. This was evaluated further by studying the behaviour of heat-polarised cells, orientated in such a way that the nucleated part was nearest to the dying red cell and the cytoplasmic pseudopod extended away from the chemotactic target (Fig. 2, 1). Various polarised cells differed in their response to chemotactic stimulation, as shown by the following examples. Occasionally a cell withdrew the distant pseudopod into the nucleated part and extended a new pseudopod towards the chemotactic red cell, as expected. More cells, however, behaved differently. The distant pseudopod migrated around the nucleated part towards the red cell, causing the nucleated section to be dragged away from the red cell, thus rotating the entire cell by 180° (Fig. 2*a*). Another cell showed a mixed response (Fig. 2*b*). Chemotactic stimulation resulted in the formation of a leading edge (metapod) on the nucleated part nearest to the destroyed red cell, as well as migration of the distant pseudopod around the nucleated part. At an early stage both sections of the cell seemed to migrate independently towards the chemotactic target. This independence of action by two parts of the cell indicated that different sections of the cell can recognise and respond separately to chemotactic stimulation. Later the more actively migrating pseudopod directed the entire cell towards the red cell, dragging the nucleated part behind it.

Fig. 1 Stages of fragment formation.



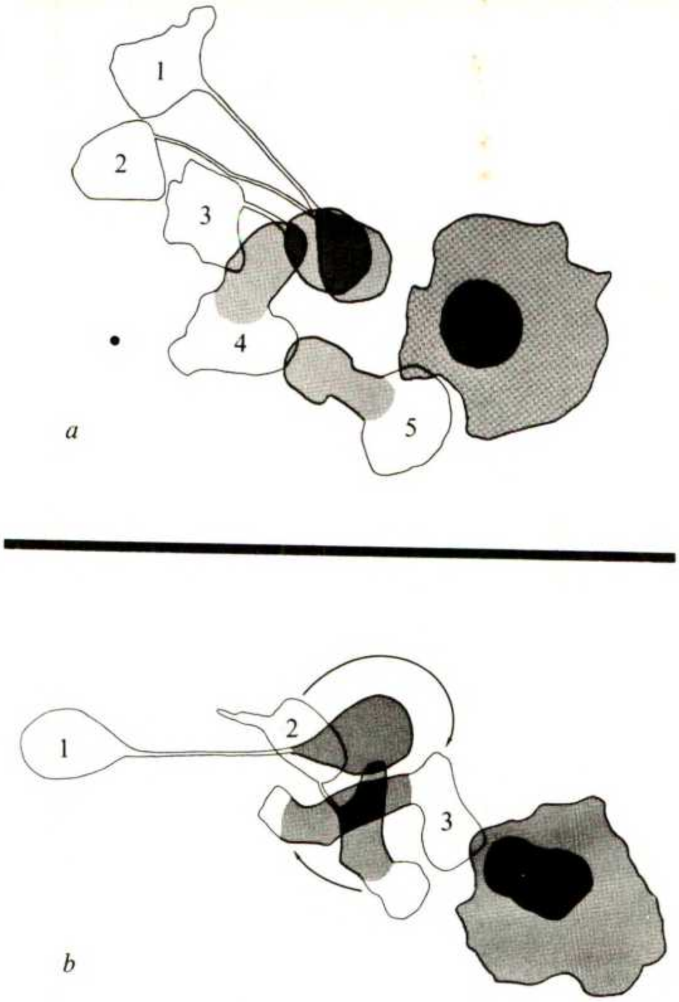


Fig. 2 Examples of polarised leukocytes responding to chemotactic stimulation: tracings of the path. Position of the leukocyte before chemotactic stimulation (1) and subsequent positions after chemotactic stimulation (2–5); nucleated part of the leukocyte (solid grey), position of intact (dark shading) and lased red cells (light shading).

Analysis of the chemotactic behaviour of heat-polarised cells reveals that these cells have different means of reorientation. There is, however, one common feature—the rearrangement of the hyaloplasm to form a leading edge (metapod). In these experiments (Fig. 2) the nucleated part often did not respond or responded to a limited extent, although this section of the cell underwent intense chemotactic stimulation. The antagonistic effect of the segmental responses observed in the cell shown in Fig. 2*b* suggests that migration is not necessarily coordinated by a single pacemaker per cell.

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Release of eosinophil chemotactic factor from human polymorphonuclear neutrophils by calcium ionophore A23187 and phagocytosis

THE number of mediators assumed to be important in the pathogenesis of acute allergic reactions has increased considerably recently. At the outset, Dale[1] suggested that histamine was the critical feature of such responses; then a role for the slow reacting substance of anaphylaxis (SRS-A) was suggested by Brocklehurst[2], one for the eosinophil chemotactic factor of anaphylaxis (ECF-A) by Kay and Austen[3,4] and more recently a kallikrein-like molecule has been described[5]. Whether preformed, like histamine, or synthesised after the immune event, like SRS-A, each of these mediators had two features in common. (1) It could be released (in primates) only after an IgE antibody–antigen interaction and (2) the cell of origin was the mast cell or the basophil. These generalisations can no longer be accepted. We have described the generation and release of SRS-A from human polymorphonuclear neutrophils (PMN) by the calcium ionophore A23187 (ref. 6), and we report here ionophore-induced release of eosinophil chemotactic factor (ECF) from that cell type. More importantly, we have been able to demonstrate that this mediator can be released by normal human PMN during phagocytosis. (We prefer to call the leukocyte-derived factor described here and in our previous publications ECF for two reasons : first, ECF is not associated only with anaphylaxis, and second, complete biochemical identity with the previously described ECF-A has not been proved.)

Table 1 Cell separation study of human leukocytes

	Lymphocyte preparation ECF (Eos. per HPF)	Lymphocyte preparation Histamine ($\mu g\ ml^{-1}$)	PMN preparation ECF (Eos. per HPF)	PMN preparation Histamine ($\mu g\ ml^{-1}$)
Buffer control	3	0.04	7	0.01
Ionophore ($5 \mu g\ ml^{-1}$)	190	0.45	1,200	0.06
a–IgE ($3 \times 10^{-2}\ \mu g\ ml^{-1}$)	150	0.38	14	0.06

HPF, high power field; Eos, eosinophils.

This table shows mediator release from 2.5×10^7 cells treated with calcium ionophore A23187 and anti-IgE. Histamine release in the PMN preparation was very low, and so was release by reverse anaphylaxis with anti-IgE. Ionophore-induced ECF release from PMNs, however, was massive. Precision of the chemotaxis assay was 15.8%. Differentials were:

	PMN	Eosinophils	Basophils	Lymphocyte + Monocyte
Lymphocyte preparation (%)	0.0	0.0	6.2	93.8
PMN preparation (%)	95.1	1.5	0.9	2.6

ECF-A is a preformed, low molecular weight peptide which can be released from various mast cell or basophil sources[3,4,7-9]. It is also released from human peripheral leukocytes by reversed anaphylaxis (anti-IgE), or with antigen exposure in the case of sensitised cells[10]. Our assay for ECF has been described before[10]. Briefly, 2.5×10^6 cells containing at least 25% human or guinea pig eosinophils were placed above a polycarbonate filter in a modified Boyden chamber, and the substance to be tested for chemotactic activity placed below the filter. After 3 h of incubation at 37 °C, the cells that have migrated completely through the filters were counted under the microscope at ×100 magnification. Antigen dose response and kinetic relationships for ECF release were identical to those for the release of histamine.

The line of investigation reported here is derived from studies with the calcium ionophore A23187, which has been shown to stimulate various secretory reactions[11,12] and to be a potent histamine-releasing agent in mast cells[13] and basophils[14]. When the ionophore was used to study the release of ECF from whole peripheral leukocyte preparations (obtained by dextran sedimentation[15]), the amount of activity obtained far exceeded that released with antigen, whereas histamine release observed was similar with both stimuli. When human basophil–mononuclear cell preparations, obtained by Ficoll–Hypaque flotation[16], were challenged with the ionophore, the release of the two mediators was similar to that obtained with antigen or anti-IgE stimulation. With purified PMN cell preparations[16], however, ionophore stimulated the release of more ECF than could be accounted for by basophil contamination. The results shown in Table 1 are representative of eight similar experiments. No lactate dehydrogenase (LDH) was released from the PMN exposed to ionphore.

The PMN-derived ECF preferentially attracts eosinophils, whether from human or guinea pig sources. Control experiments demonstrated that the concentrations of ionophore used experimentally did not cause chemotaxis, nor did the ionophore generate further chemotactic activity in ECF containing cell-free supernatants. Chromatographic analysis of the PMN-derived ECF on Sephadex G-25 demonstrated a single peak of eosinophil chemotactic activity with a molecular weight of approximately 600, identical to that found in chromatographic analysis of antigen or anti-IgE-induced ECF-A from purified basophil preparations.

Having established that ECF could be produced by human PMN, we investigated if it could be released by physiological stimuli. Since phagocytosis leads to the secretion of a number of enzymes[17,18], we studied this phenomenon for the release of ECF from cells. As a phagocytic stimulus, we used a standard preparation of zymosan particles treated with normal human serum, (Z(x)), that is, coated with complement factors and then washed extensively. The zymosan particles and human PMN were incubated together at 37 °C and the supernatants were removed by centrifugation at different times and checked for ECF

Table 2 Kinetic studies of ECF generation

Time (min)	βG (Δ *A*)	ECF (Eos. per 5HPF)	LDH	NBT (Δ *A*)
10	0.052	1,325	Baseline	0.110
30	0.070	1,550	Baseline	0.130
90	0.070	200	Baseline	0.130

Z(x) (1.5 mg) was incubated with 2.5×10^6 PMN in a final volume of 1 ml. After various times the mixture was centrifuged, and the supernatants assayed for chemotactic activity. For β-glucuronidase (βG) determinations, 100 µl per ml of the supernatant was incubated with 0.01 M phenolphthalein glucuronic acid and 0.5 ml of 0.1 M acetate buffer, *p*H 5.0, for 12 h. Absorbance (*A*) was read at 540 nm for βG and at 360 nm for LDH. (Δ*A* represents the reading at each time, minus cell and buffer control.) For the NBT test, extracted formazan was read at 515 nm. Supernatants of Z(x) alone did not show chemotactic activity. Values obtained were comparable in four different experiments.

activity. β-Glucuronidase and LDH release were measured by standard techniques[19], as was the nitroblue tetrazolium (NBT) reduction of the cells during phagocytosis[20].

As Table 2 shows, release of the lysosomal enzyme marker β-glucuronidase parallelled phagocytosis as indicated by NBT reduction; cell viability was maintained since there was no release of the cytoplasmic enzyme marker LDH. Phagocytosis also led to highly significant ECF release, which at first parallelled the release of β-glucuronidase, but by 90 min, the ECF activity in the supernatant was markedly decreased.

The chemotactic factor released by phagocytosis was as highly selective for guinea pig and human eosinophils as that derived by ionophore stimulation. Sephadex G-25 chromatography revealed an apparent molecular weight of 600, similar to the ECF preparations described here. The ECF therefore seems to be distinct from the non-dialysable chemotactic factor described by Zigmond and Hirsch[21] generated from human PMN during phagocytosis of aggregated gamma globulin. This latter factor also differs in that, unlike ECF, it attracts neutrophils as well as eosinophils.

Kinetic studies of ECF generation (Table 2) during phagocytosis indicated that, after reaching a peak, the chemotactic activity decreased rapidly. Results of preliminary experiments suggest that this decrease results from the destruction of ECF. Sonication of purified PMN led to little or no eosinophil chemotactic activity, but when sonicates were mixed with supernatants obtained from phagocytosing cells, there was a distinct reduction of chemotactic activity. The nature of the material(s) which caused destruction or inactivation of ECF in sonicates and during the late phase of phagocytosis is under study.

The data reported here indicate that ECF is present in human PMN and that it is released during phagocytosis. This finding is novel in that this mediator of the allergic response was thought to exist only in mast cells and basophils and to be released only after IgE–antigen interaction. Because PMN are present in the human body in much greater numbers than either basophils or mast cells, the quantitative contribution of these cells to inflammatory events is likely to be of great importance. These rather surprising observations further blur the artificially drawn lines between the various types of inflammatory processes. If the same mediators can be released by IgE-mediated processes from basophils, or from PMN by phagocytosis, the differentiation between these processes becomes more subtle and complex.

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Role of stem cell migration in initiation of mouse foetal liver haemopoiesis

THEORIES of the development of mammalian hepatic haemopoiesis have polarised around two viewpoints. One hypothesis suggests that multipotential haemopoietic stem cells (HSC) migrate from the yolk sac and colonise the developing hepatic primordia[1]. According to the other view, hepatic haemopoietic tissue arises from the transformation of liver mesenchyme cells and thus has no direct relationship with vitelline haemopoiesis[2]. To solve this developmental problem, it is necessary to establish techniques, either *in vivo* or *in vitro*, to maintain hepatic tissue in a foetal state. *In vitro* studies have demonstrated that hepatic haemopoietic tissue can develop if tissues are explanted after the 28-somite stage[3]. Thus, these experiments did not verify the inductive model[2] of hepatic haemopoiesis, although the *in vitro* conditions may have curtailed haemopoietic induction without altering hepatic differentiation. With an exogenous source of yolk-sac-derived HSC attempts have been made to colonise pre-28-somite hepatic explants *in vitro*, using the appearance of granulopoiesis as an indication of successful recombination. These experiments were not conclusive since granulopoiesis

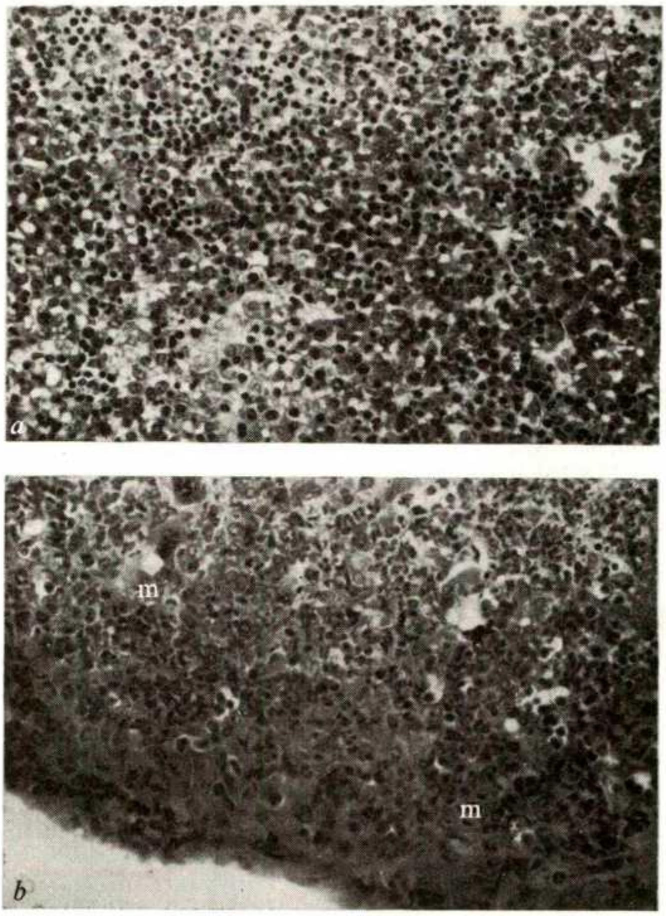

Fig. 1 *a*, Section of 15-d CBA foetal liver, erythroid differentiation predominates (×168). *b*, Section of 13-d CBA foetal liver, sectioned after being grafted on to kidney for 2 d. Granulocytes in various stages of differentiation are apparent. Mitotic figures (m) can also be observed (×168).

was observed with yolk sac cells *in vitro* in the absence of hepatic tissue (G.R.J., unpublished).

To overcome some of these problems we have used an *in vivo* grafting technique. Hosts were prepared in various ways, either being non-irradiated, irradiated (850 r.) or partially hepatectomised; they included BALB/c *nu*/*nu* (nude), (CBA × C57BL) Sl/Sl^d, CBA and (CBA × C57BL) F$_1$ mice. Tissues for grafting were obtained from foetuses of the CBA or (CBA × C57BL) F$_1$ strains. Females of known gestational age (day of vaginal plug discovery equals day zero of gestation) were killed by cervical dislocation and foetal hepatic tissue was removed aseptically as before[3]. Intact hepatic tissue, obtained from foetuses younger than 11.5 d of gestation (36 somites), was placed under the kidney capsule of syngenic 8-week-old mice. With older hepatic tissue, portions not larger than 2 mm^3 were placed on to a graft bed prepared by shaving off a thin slice of host renal cortex[4]. One graft per host was our standard procedure. After suitable intervals, hosts were killed and the kidneys with attached grafts were removed, fixed in Bouin's fixative, sectioned and stained with haematoxylin and eosin.

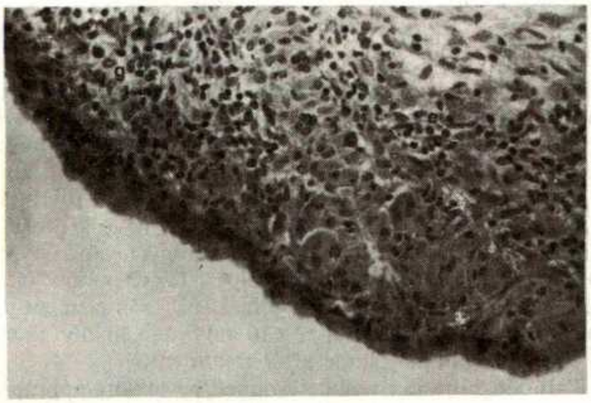

Fig. 2 Section of 24-somite CBA foetal liver graft after 7 d under the kidney capsule of a Sl/Sl^d host. g, Granulocytes; e, erythroblasts (× 168).

With all grafts that were initially older than 28 somites, haemopoiesis declined rapidly and 10 d after grafting only some connective tissue remained, surrounded by extensive necrotic areas. Cell suspensions derived from the grafts were counted and subjected to differential cell counts. *In vivo* spleen colony assay for HSC and the *in vitro* agar assay for granulocyte–macrophage progenitors (CFC) were performed as before[1]. All these parameters verified the haemopoietic decline, HSC and CFC were practically absent by 6 d and at the same stage the cellularity had been reduced by 90%. In all host combinations a marked morphological change was observed in the graft tissue, the predominately erythroid tissue[5] becoming granulocytic (Fig. 1). This subjective view was verified by differential cell counts on smears derived from graft cell suspensions; up to 44% of the haemopoietic cells within 2 d of being grafted were of the granulocyte lineage (Table 1). Numerous mitotic figures were also observed within the masses of granulocytes (Fig. 1).

Table 1 Percentage figures for erythroblasts and granulocytes from normal 13-d and 15-d CBA foetal livers and of 13-d CBA foetal liver grafted on to host kidney cortex for 2 d (15 d equivalent).

	Erythroblasts (%)	Granulocytes (%
13-d gestation CBA foetal liver	88	8.25
15-d gestation CBA foetal liver	92.75	5.5
13-d gestation CBA liver, 2 d in irradiated F$_1$ host	20.5	44.6
13-d gestation CBA liver, 2 d in non-irradiated F$_1$ host	41.25	30.75

Figures obtained from smears, 500 haemopoietic cells scored, numbers of erythroblasts and granulocytes are expressed as a percentage of the total number of haemopoietic cells.

With hepatic grafts younger than the 28-somite stage, some hepatic differentiation appeared to continue, even when sampled 10 and 12 d after grafting. Morphological integrity was maintained in these grafts and by 10 d duct systems had developed, necrotic areas being virtually absent. At no stage, however, was haemopoietic tissue observed in pre-haemopoietic grafts that had been placed in non-irradiated, irradiated, nude or partially hepatectomised hosts. With Sl/Sl^d hosts, however, small areas of erythroid and granuloid tissue were observed when grafts were sampled after a minimum period of 7 d (Fig. 2). In this situation grafts as early as the 22-somite stage displayed some haemopoietic areas.

These results may be interpreted as indicating than an exogenous supply of haemopoietic cells is necessary for the establishment of haemopoietic tissue in the developing foetal liver. When no hameopoietic tissue developed, the host either had no HSC (irradiated hosts) and therefore could not supply the graft with cells, or had normal haemopoietic tissue (non-irradiated, partially hepatectomised or nude hosts) and therefore presumably no requirement for extra-haemopoietic areas. In contrast Sl/Sl^d mice are anaemic but when transplanted with exogenous haemopoietic tissue, they support haemopoiesis. Further substantiation for the need of an exogenous source of haemopoietic cells for the initiation of hepatic haemopoiesis was gained from the following experiments. Hosts bearing post-28-somite hepatic grafts were irradiated (850 r.) and then reconstituted with 10^7 syngeneic bone marrow cells. Seven days after irradiation-reconstitution grafts displayed prominent erythoid, megakaryocytic and granuloid colonies, the latter being the more frequent (Fig. 3). Control grafts placed in hosts subsequently irradiated but not grafted with bone marrow did not reveal haemopoietic colony formation.

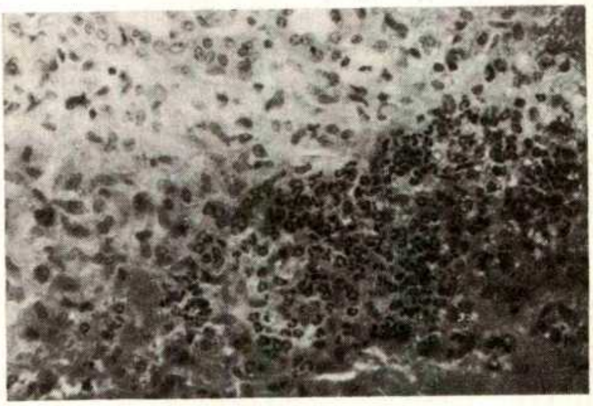

Fig. 3 Granulocyte colony in graft of 16-d CBA foetal liver, irradiated after grafting, and reconstituted with syngeneic bone marrow cells on day of grafting. Section sampled 7 d after grafting (× 168).

Although haemopoiesis did not persist in hepatic grafts, observations made during these experiments are interesting. The mouse foetal liver is predominately erythroid with up to 90% of the haemopoietic cells in 13-d gestation animals differentiating along this pathway[5]. Since the incidence of CFC in foetal hepatic tissue is as high or higher than in adult marrow[6] the lack of extensive granulopoiesis suggests that either microenvironmental or external regulatory factors suppress this line of differentiation. In our experiments foetal hepatic tissues were grafted into adult hosts and any external foetal inhibitory factors may have been bypassed, thus allowing granulocyte expression. This removal of inhibition may also be reinforced by colony stimulating factor (CSF) present in normal mouse sera[7]. Therefore, with elevated levels of this substance a further increase in granulopoiesis would be expected. Such an increase of CSF has been reported in the serum of irradiated mice[8] and in our experiments grafts placed into irradiated hosts contained higher percentages of granulocytes than grafts derived from non-irradiated hosts (Table 1).

Supplementing the above proposed humoral affects, alterations in the types of haemopoietic inductive microenvironments[9] in the grafts could produce the observed results. Thus the increased granulopoiesis may be accounted for by a loss of the erythroid-inducing microenvironment and a stabilisation or increase in the numbers of granulocyte inductive microenvironments.

The fact that pre-haemopoietic foetal liver can support haemopoiesis if supplied with an exogenous source of haemopoietic cells supports the cell-seeding hypothesis[1]. The absence of any visible haemopoietic tissue in pre-haemopoietic hepatic grafts, placed into irradiated hosts, seems to invalidate the theory of induction[2]. It is possible, however, that the experimental procedure prevents the inductive event or alternatively that the pre-haemopoietic grafts rapidly lose the factors necessary for the maintenance and subsequent differentiation of HSC. This latter criticism is opposed by the observation that hepatic grafts, which were irradiated after placement on the host kidney, could still produce discrete colonies even if the host was reconstituted 3 d after irradiation. The results reported here therefore suggest that an exogenous supply of HSC is necessary for the development of hepatic haemopoietic tissue. After the attachment of these cells, local microenvironmental and/or external humoral factors then determine their subsequent differentiation.

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Cooperation across the histocompatibility barrier

STUDIES in mechanisms of T–B collaboration in the immune response have shown that T cells can produce factors after stimulation with antigen[1–3], allogeneic cells[4–6], or mitogens (ref. 7 and O. Sjöberg, unpublished), that can stimulate B cell responses to antigens. Such factors have been categorised as specific[1,3] or nonspecific (refs 4–7 and O. Sjöberg, unpublished). Studies have shown that the specific factor produced by (T,G–A—L) stimulation of educated T cells[8] is able to promote antibody formation by both syngeneic and allogeneic cells. Similarly, with one exception[8], nonspecific factors from antigen- or mitogen-activated T cells were able to cooperate with B cells from histoincompatible strains[7,9]. Reports that histoincompatible T and B cells could not be demonstrated to cooperate in a humoral immune response[10], in conditions in which it was considered that inhibitory allogeneic effects were excluded or irrelevant, indicated that for efficient collaboration T and B cells need to share surface determinants coded for by major histocompatibility complex (MHC), in particular the I region or its equivalent. One report[11] argued against this; allophenic mice (C3H+C57BL) were able to make non-responder allotype towards (T,G)–A--L. This finding made the presumption that it was the high responder T cells which were collaborating with the low responder B cells, but could not exclude that alternative cooperative mechanisms were operational.

The present studies represent a direct analysis using T cells derived from bone marrow chimaeras[12] and testing them with histocompatible B cells. In this situation, as in allophenic mice, the T cells are rendered specifically unresponsive to the determinants of the H–2 complex of the B cells used in testing for collaboration.

B cells were obtained from anti-θ treated spleens of CBA (H–2^{k}), BALB/c (H–2^{d}) or C3H×BALB/c (H–2^{k}×H–2^{d}) F$_1$ mice which had been primed at least 3 weeks previously with 500 μg trinitrophenyl-fowl gamma globulin (TNP–FGG) in Freund's complete adjuvant (FCA). Helper T cells were derived from the spleens of mice primed with 500 μg KLH in FCA 3 weeks, and again with 50 μg soluble keyhole limpet haemocyanin (KLH) 3 d before collection. B cells were in large part removed by passage through nylon wool, and red cells were lysed by treatment with 0.17 M ammonium chloride. Bone marrow chimaeras (C3H+BALB/c) (H–2^{k}+H–2^{d}) were derived by transfer of anti-θ-treated bone marrow cells from C3H and BALB/c donors to irradiated F$_1$ recipients. Mice were primed with KLH to generate helper cells at least 3 months after bone marrow transfer.

Anti-H–2^{k} (B.10 anti-A/J) and anti-H–2^{d} (B.10 Br anti-DBA/2) sera were absorbed with either BALB/c or C3H lymphocytes to render them specific for only one type of cell in the chimaera. Where helper T cells were treated with these alloantisera and guinea pig complement they were initially suspended at 2×10^7 ml^{-1} and finally adjusted to the same initial volume after treatment.

T–B collaboration was determined by mixing appropriate combinations of KLH-primed T cells with anti-θ-treated TNP–FGG-primed B cells in 10 μl volume in the wells of Terasaki plates[13] in the presence of 2–5 μg ml^{-1} TNP–KLH. After 5 d incubation at 37 °C, 30 wells from each treatment group were sampled for anti-TNP plaque-forming cells (PFCs) by conventional plaquing techniques using TNP coupled to Donkey erythrocytes. Results are displayed as the number of responding cultures and the total anti-TNP PFCs derived from 30 wells.

Table 1 shows results of an experiment which confirms the finding[10] that non-tolerant histoincompatible T and B cells will not cooperate *in vitro*. CBA (H–2^{k}) T cells

Table 1 Total (direct and indirect) PFC responses (1.5×10^5 per well) of anti-θ-treated TNP–FGG primed spleen cells to 2 μg ml^{-1} TNP–KLH in presence of histocompatible and histoincompatible KLH-primed T cells

B cell source	T cells source (No. per well)	No. of positive wells (30 wells assayed)	Total PFCs (direct + indirect PFCs, 30 wells)
BALB/c	BALB/c		
	9×10^5	8	100
	4.5×10^5	13	230
	CBA		
	9×10^5	2	22
	4.5×10^5	2	17
CBA	BALB/c		
	9×10^5	3	22
	4.5×10^5	1	7
	CBA		
	9×10^5	28	871
	4.5×10^5	29	795

Table 2 Ability of histoincompatible T cells ($H-2^d$) derived from a chimaera to cooperate with $H-2^k$ B cells

B cells	T cells	Antiserum treatment	No. of positive wells (30 wells assayed)	Total anti-TNP–PFC in 30 wells	% Dead T cells
C3H×BALB/c F_1 ($H-2^k \times H-2^d$)	F_1 ($H-2^k \times H-2^d$)	None	30	8,300	12
	Chimaera ($H-2^k + H-2^d$)	None	30	4,245	15
	Chimaera	Anti-$H-2^k$	30	6,484	57
	No T cells	—	0	—	—
CBA($H-2^k$)	F_1	None	30	3,469	12
	Chimaera	None	30	2,939	15
	No T cells	—	1	12	—
	F_1	Anti-$H-2^k$	2	31	94
	Chimaera	Anti-$H-2^k$	30	3,796	57

B cells were from either anti-θ-treated C3H×BALB/c or CNA TNP–FGG-primed spleen cells. T cells were derived from nylon wool-enriched KLH-primed spleen cells. Anti-$H-2^k$ treatment of T cells was carried out by incubating 2×10^7 cells with 1 ml 1:5 dilution of anti-$H-2^k$ antiserum for 30 min on ice, and subsequently with 2 5 ml 1:6 GPC for 30 min at 37 °C. T cells were then spun through foetal calf serum and resuspended to 1 ml. All cultures were challenged with 5 μg ml^{-1} TNP–KLH, and anti-TNP responses determined after 5 d.

cooperate efficiently with CBA B cells but not with BALB/c cells. Similarly, it is clear that BALB/c T cells ($H-2^d$) cooperate best with BALB/c cells, and poorly with CBA B cells We have found it persistently difficult, however, to achieve good responses with BALB/c cells in this system.

This inability of $H-2^d$ cells to function with $H-2^k$ B cells is not seen if $H-2^d$ T cells are derived from an ($H-2^k$+$H-2^d$/BALB/c+C3H) chimaera. Table 2 shows the results of an experiment in which anti-$H-2^k$ alloantiserum-treated chimaeric cells cooperate at least as efficiently as untreated T cells with either of ($H-2^k \times H-2^d$) or $H-2^k$ B cells. The efficacy of the anti-$H-2^k$ treatment is clearly demonstrated by the fact that F_1 T helper cell function was eliminated by identical anti-$H-2^k$ treatment. The confirmation that it was truly the remaining $H-2^d$ T cells which were collaborating with the $H-2^k$ B cells is shown in Table 3, in which the helper capacity of anti-$H-2^k$-treated chimaeric cells was totally abrogated by further treatment with anti-$H-2^d$ alloantiserum. Furthermore, it was clear that the responding B cells were not contaminating syngeneic cells in the populations enriched with T cells, as these did not respond to TNP–KLH in the presence of irradiated CBA spleen cells These data also indicate that the frequencies of helpers from $H-2^d$ cells for $H-2^k$ B cells must also be in the range found with the syngeneic cells.

Table 3 Ability of histoincompatible T cells ($H-2^d$) to cooperate with $H-2^k$ B cells

B cells	T cells	Antiserum treatment	Number of positive wells (30 wells assayed)	Total anti-TNP–PFC in 30 wells	% Dead T cells
CBA ($H-2^k$)	F_1($H-2^k \times H-2^d$)	None	30	4,464	10
	Chimaera $H-2^k + H-2^d$)	None	30	3,459	18
	CBA ($H-2^k$)	None	30	5,730	3
	No T cells	—	0	0	—
	CBA	Anti-$H-2^d$	30	4,046	3
	F_1	Anti-$H-2^d$	13	578	58
	F_1	Anti-$H-2^k$	5	179	85
	F_1	Anti-$H-2^k$+$H-2^d$	2	25	95
	Chimaera	Anti-$H-2^k$	30	5,220	55
	Chimaera	Anti-$H-2^k$+$H-2^d$	0	0	93
CBA spleen cells irradiated with 1,200 r.	Chimaera	None	1	38	
	F_1	None	1	36	
	CBA	None	2	40	

B cells and T cells were prepared as in Table 2. Antiserum treatment was as in Table 2 except that some chimaeric and F_1 T cells were treated with anti-$H-2^k$ (1 ml 1:5) and anti-$H-2^d$ (1 ml 1:2) sequentially before incubation with GPC. All cultures were challenged with 5 μg ml^{-1} TNP–KLH and responses determined after 5 d Viability data in this Table and in Table 2 confirm the chimaeric nature of the presumed chimaeric mice.

Our data are in agreement with the recent *in vivo* findings of von Boehmer *et al.*[14], who showed that SRBC-primed T cells from chimaeras would also cooperate with histoincompatible B cells for an anti-SRBC response on adoptive transfer, and also support the findings[15] that alloantigens of the B cell donor will also collaborate efficiently in an *in vitro* primary response of rat spleen cells. Similar results have also been obtained by negative selection procedures in the mouse (J. Quintans, unpublished). It is relevant that our data deal with the responses to TNP–KLH; unlike SRBCs the soluble form of this antigen cannot be helped by allogeneic effects or nonspecific factors[16] which cannot be totally excluded in the data of Heber-Katz and Wilson. The original experiments of Katz *et al.*[10] concerning requirements for histocompatibility for effective collaboration similarly used TNP–KLH in an equivalent *in vitro* system.

In conclusion, we can find no obligatory requirement for identity at the MHC for effective T–B collaboration in the *in vitro* secondary immune response. This does not exclude, however, any more subtle requirement for MHC identity between cells for the generation of efficient helpers.

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Low dose unresponsiveness with a thymus independent antigen

LIPOPOLYSACCHARIDES (LPS) from Gram-negative bacteria are very efficient carriers for haptenic determinants, so that B cells can be activated by such conjugates to produce antihapten responses both *in vivo* and *in vitro* in the absence of T helper cells[1-3] and macrophages (our unpublished data). Trinitrophenylated LPS (TNP–LPS) is one such thymus-independent antigen. Most antigens of this category can induce unresponsiveness in animals only when administered in high concentrations[4], unlike certain thymus-dependent antigens which can induce unresponsiveness *in vivo* in both low doses (low zone unresponsiveness) and high doses (high zone unresponsiveness)[5]. In the case of low zone unresponsiveness to thymus-dependent antigens, only helper T-cell functions seem to be affected[6]. It was therefore interesting when we observed that very low doses of LPS could render mice unresponsive to subsequent TNP coupled to LPS. Only in one previous case has low zone unresponsiveness to a thymus-independent antigen been observed, and this was with pneumococcal polysaccharide (SSS III) through a mechanism involving suppressor T cells[7]. We report here that low zone unresponsiveness achieved with LPS is highly specific and requires no obvious T-cell-mediated suppressor mechanisms, thus indicating a further type of regulation mechanism for antibody responses. As LPS are intimately related to other bacterial surface antigens it may be that similar mechanisms operate in the determination of natural immunological reactions against Gram-negative bacteria.

Table 1 T-cell independence of unresponsiveness

Groups*	PFC per 10^7 spleen cells *nu/nu*	 Hairy littermates
Controls	4,783	7,292
Preinjected with LPS (0.07 µg)	390	353

Spleens from seven *nu/nu* and five littermates were each assayed in duplicate. Arithmetic means are shown.

*Mice were injected with 5 µg of TNP–LPS 5 d after injection of water (controls) or 0.07 µg LPS.

CBA, C3H, C3H/HeJ, BALB/c and BALB/c *nu/nu* mice, bred in the Department of Pathology, Cambridge were used. Recipients for transferred spleen cell inocula were irradiated with 675 rad from a Co source. Trinitrophenylated (TNP) derivatives of these LPS (*Escherichia coli* 055:B5) and LPS (*Serratia marscescens*) were obtained from Difco and were prepared by the technique of Jacobs and Morrison[1]. Dinitrophenylated (DNP)–dextran was a gift from Dr C. Desaymard. All injections were given intraperitoneally, and all plaque-forming cells (PFC) were assayed[8] by conventional techniques. Anti-TNP responses were measured at the optimal time, 3 d after challenge, except where stated otherwise. Initial experiments showed that when BALB/c mice were injected with various doses of LPS (*E. coli*) 5 d before challenge with TNP–LPS as little as 7×10^{-4} µg LPS could diminish the subsequent anti-TNP response to 36% of the control level and 7×10^{-2} µg effectively abolished it (our unpublished data). This effect could be seen 72 h after treatment with 7×10^{-2} µg LPS and was complete by 96 h (our unpublished data). The phenomenon does not obviously depend on any T-cell suppressor mechanisms as it is seen in nude mice (Table 1), but it is carrier specific in that none of the responses to DNP–dextran, TNP–LPS (*S marscescens*) or sheep red blood cells (SRBCs, Table 2) were affected. This suggests that the specificity of the unresponsiveness is related to the polysaccharide portion of O antigens of LPS as these seem to be the immunodominant determinants.

Table 3 Abrogation of unresponsiveness after lymphocyte transfer

Pretreatment of donors of nude spleen cells	Pretreatment of irradiated recipient mice*	TNP–PFC per spleen†
None	None	10,900
LPS (0.07 µg) on day −5	None	12,040
None	LPS (0.07 µg) on day −5	200

*Heterozygous BALB/c littermates were irradiated with 600 rad. Nude spleen cells (40 × 10^6) were then transferred to recipients. TNP–LPS (*E. coli*) (5 µg) were injected 2 h later.

†Each value represents the geometric mean of PFCs per spleen from four mice each assayed in triplicate.

The status of TNP-specific B cells seemed to be unaffected by LPS (*E. coli*) because spleen cells from treated groups responded as well as untreated controls in adoptive transfer. In contrast mice pretreated with LPS (*E. coli*)

Table 2 Specificity of low dose unresponsiveness

Treatment of mice (CBA),	 DNP–Dextran	PFC per spleen from challenge with TNP–LPS (*E. coli*)	 SRBCs	 TNP–LPS (*S. marscescens*)
Experiment 1				
LPS (*E. coli* 055: B5) (0 07 µg) on day −5	21,000	1,000	17,720	NT
Nil	21,050	74,000	11,140	NT
Experiment 2				
Nil		50,000*		33,150*
LPS (*E. coli*) (0.07 µg) on day −5		3,970*		41,110†

DNP–Dextran (2.5 µg) was given 4 d before TNP–PFCs were assayed. TNP–LPS (2.5 µg) was given 3 d before TNP–PFC were assayed. SRBCs (0 2 ml, 5%) were given 4 d before SRBC–PFC were assayed. Figures represent the geometric mean of the response of three to six spleen donors. NT, not tested.

*TNP–LPS (10 µg) was injected into each mouse. TNP–PFC were assayed 3 d later.

†Each value represents the geometric mean of PFCs per spleen from four mice assayed in triplicate.

were poor recipients for adoptively transferred normal spleen cells in that they responded poorly to TNP–LPS (*E. coli*) but normally to TNP–LPS (*S. marscescens*) (Table 3). Further unpublished data show that LPS non-responder mice (C3H/HeJ) pretreated with LPS behave in the same way as untreated recipients for normal cells (that is they produced no suppression). Therefore, unresponsiveness must be related to a lipid A-dependent activation event[8–10], the most likely of which could be the production of anti-LPS antibody, albeit in very small amounts.

In conclusion our data demonstrate that a highly immunogenic thymus-independent antigen, LPS, generates, on low dose priming, an LPS-specific product which prevents the successful interaction of TNP–LPS with hapten-specific cells We predict that this phenomenon should not be exclusive to LPS but should be detectable with other strongly immunogenic thymus-independent antigens

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Effect of tumour necrosis factor on cultured human melanoma cells

CARSWELL *et al*[1] described a tumour necrosis factor (TNF) in the sera of mice sensitised with BCG and injected with endotoxin. After intravenous injection of this serum into mice bearing a 7-d-old transplant of a tumour originally induced by methylcholanthrene, necrosis of the tumour began within 12–24 h. A similar response was found in mice bearing various other tumours. We have now found that both whole TNF serum and TNF partially purified from mouse serum[2] inhibit the growth of a human melanoma cell line.

We have screened neuroblastoma and melanoma cell lines to determine their susceptibility to TNF. We used these tumours because they have a high degree of spontaneous[3] or immune-induced regression[4]. They had all been maintained in continuous culture in our laboratories and consisted of a melanoma (RPMI 7931) originally established by George E Moore, Roswell Park Memorial Institute and maintained free of mycoplasma by Dr Jørgen Fogh, Memorial Sloan–Kettering Cancer Center; three neuroblastoma lines, two described previously[5], and a fibroblast line derived from the bone marrow of a child with neuroblastoma

TNF in serum or after partial purification[2] (fraction G-200 II) had no growth inhibitory or toxic effects on neuroblastoma or fibroblast cells. These observations agree with those of Carswell *et al.*[1], who found that normal mouse embryo fibroblasts (MEF) were insensitive to TNF. In contrast, growth of melanoma cells was inhibited (Table 1) At the highest level of TNF activity, that is where the concentration of protein was 0.45 mg ml^{-1} or more, cell proliferation stopped. When the concentration of TNF was decreased, the extent of inhibition of cell growth was

Table 1 Effects of TNF on growth *in vitro* of melanoma cells (RPMI 7931)

Substance added to culture medium	Mouse serum protein in culture medium (mg ml^{-1})	Inhibition of growth (%)
(1) None	0	0
(2) Serum (untreated mice)	7.0	0
(3) Serum (endotoxin-treated mice)	7.0	0
(4) Serum (BCG-treated mice)	7.0	0
(5) Serum (BCG+endotoxin-treated mice)	7.0	100
(6) G-200 II from sera of normal mice	0.2	0
(7) G-200 II from sera of BCG+endotoxin-treated mice (TNF)	0.05	10
(8) G-200 II from sera of BCG+endotoxin-treated mice (TNF)	0.1	10
(9) G-200 II from sera of BCG+endotoxin-treated mice (TNF)	0.2	75*
(10) G-200 II from sera of BCG+endotoxin-treated mice (TNF)	0.45	100
(11) G-200 II from sera of BCG+endotoxin-treated mice (TNF)	1.0	100
(12) None*	0.0	80

*Cells from this culture were replated without additional TNF (see line 12).
G-200 II represents a 20-fold purification of TNF[2].

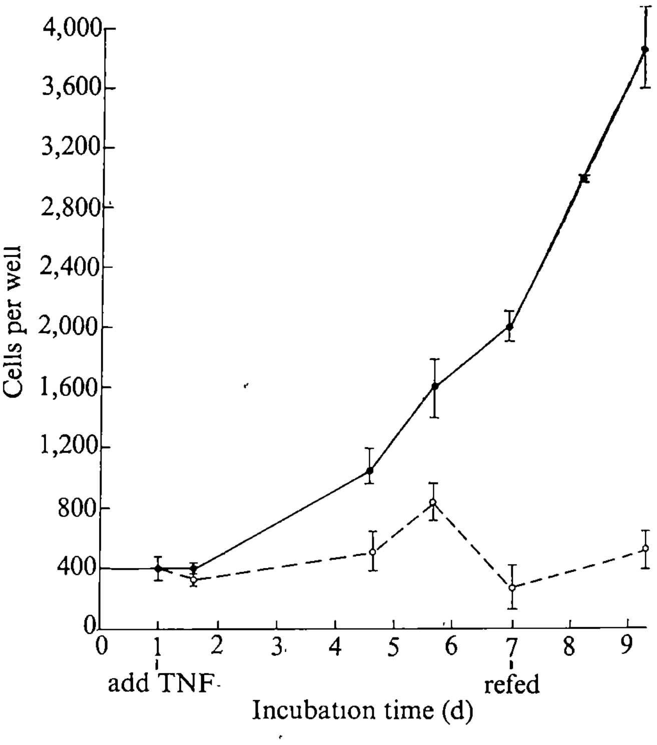

Fig. 1 Melanoma cells were grown in 25-ml plastic flasks (Falcon) containing 7 ml of Eagle's medium with glutamine, non-essential amino acids, penicillin, streptomycin and 15% heat-inactivated foetal bovine serum. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere. Stock cultures were grown to confluence and refed with fresh medium, 24 h later the cells were detached with 0.25% trypsin and 500–1,000 cells in 0.1 ml were inoculated into wells of a 96-well flat bottomed microtitre plate. To each test well 0.06 ml of a solution of TNF was added. The final concentration of the TNF protein per ml of incubation mixture ranged from 0.05 to 1.0 mg. At 2–3-d intervals the number of cells from three control and three TNF-treated wells were determined as follows. The well contents were removed, each well was washed twice with phosphate-buffered saline and 0.2 ml of a 0.25% trypsin solution was added to detach adhering cells. After 15 min the total number of cells in the suspension, ranging in size from 80 to 280 µm, was counted with a Coulter counter. ●, Normal medium; ○, medium plus TNF (1.0 mg per ml^{-1} mouse serum protein).

reduced. Inhibited cells which were replated in fresh media without additional TNF (Table 1, lines 9 and 12) did not return to their normal growth rate. The kinetics of melanoma cell growth in the presence or absence of TNF are shown in Fig. 1.

This inhibition of human melanoma cells by partially purified TNF was not due to nonspecific cytotoxic material present in mouse serum, for crude and identically prepared fractions from normal mouse serum lacked growth inhibitory activity. The persistence of growth inhibition after replating suggests that (1) the factor might remain bound to the cell; (2) plating efficiency is diminished secondarily to structural alterations in the cell membrane caused by contact with TNF, or (3) TNF induces metabolic alterations within the cell.

In summary, the combination of BCG sensitisation and endotoxin challenge in mice results in the production and release into their circulation of a substance which is cytotoxic to cultured human melanoma cells.

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4a(W4) and 4b(W6) human histocompatibility antigens are specifically associated with complement receptors

THE biological role of the histocompatibility genes is currently the subject of intense research and speculation[1–3]. It has been shown that efficient cell cooperation and thus immune response requires H–2 (I region) compatibility[4] and that some components of the complement (C) system, including complement receptors (CR) which may have an important role in cell–cell interaction and in lymphocyte cooperation[5,6] are also under the control of the MHS both in mouse[7] and man[8,9]. In mouse, we have shown that the CR polymorphism is partly controlled by genes mapping in the I-C or S region of the H–2 complex[10] Now, in man, we show also that the cell surface gene products of the diallelic human HLA 4a, 4b system[11] (also named W4 and W6, respectively)—now established to be an independent[12] genetic system inside the HLA complex and whose gene products are believed by some to be HLA–B basic[12] or supertypic[13] specificities—could form an integral part of complement receptors. In short anti-4a and anti-4b antisera (or their F(ab′)$_2$ fragments) are able specifically to block the rosetting capacities of complement receptors on 4a and 4b positive lymphocytes respectively (that is with antibody (A) and complement (C)-coated sheep red blood cells (E), (EAC)).

Blood samples were obtained from normal people and some chronic lymphatic leukaemia patients who had previously been typed for HLA–A, –B, –C and 4a, 4b antigens. The blood was defibrinated, layered on Ficoll–Triosil and centrifuged. The lymphocyte band was removed and washed in veronal buffer solution (VBS); the cells were resuspended in VBS, subsequently divided into aliquots and preincubated with anti-HLA–A, –B and –C antisera and anti-4a, 4b antisera or their F(ab′)$_2$ fragments. After washing twice in VBS, complement receptors were assayed by a modification of the rosetting method described in ref. 14. The rosettes were counted under a fluorescent microscope after the addition of 0.01% acridine orange to the suspensions. Pretreatment of homozygous 4a cells with anti-HLA–A –B and –C antibodies (Table 1) and anti-4b sera did not affect the number of complement rosetting cells (CRC). In contrast, both the anti-4a antisera used inhibited by 60–70% the number of complement rosettes formed. Similar results were obtained using anti-4b sera with homozygous 4b cells The number and variety of HLA antisera and the various cell types used make it unlikely that serum activity other than anti-4a, 4b antibodies was responsible for CR inhibition. The results with 4a/4b heterozygous lymphocytes were more complex. Complement rosettes made with these cell suspensions (except with BR lymphocytes) were more easily inhibited by anti-4b, than anti-4a sera. Since by cytotoxic definition most heterozygous 4a/4b individuals' lymphocytes express both 4a and 4b antigens; because of the difference in susceptibility to inhibition by the anti-4a, anti-4b antisera by different individuals' lymphocytes, we find that it is possible to subdivide the heterozygotes as follows: (1) those with CR only inhibited by anti-4b (BW; KH); (2) those with CR most readily inhibted by anti-4a, but also significantly inhibited by anti-4b (BR) and (3) those with CR most readily inhibited by anti-4b, but also significantly with anti-4a (CB, JP). When anti-4a and anti-4b were both used at the

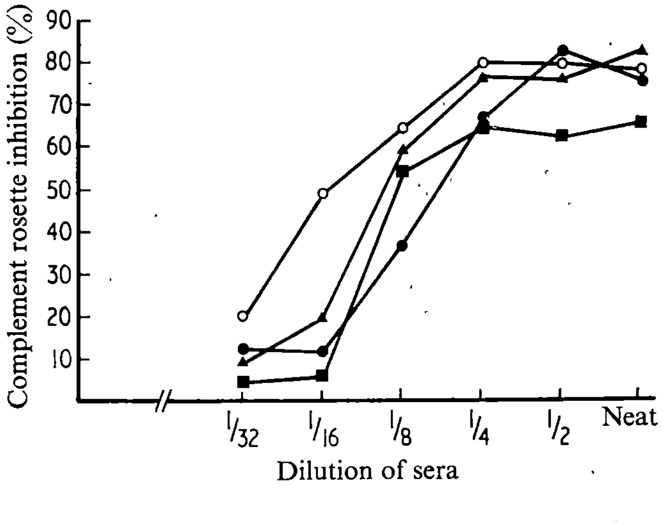

Fig. 1 For titration of anti-4a sera, AA-V cells were used; for titration of anti-4b sera, MW cells were used (see Table 1). The working dilutions used throughout were: for both anti-4a (1:2); for both anti-4b (1:4). Lower dilutions produced cell agglutination. These particular 4a, 4b antisera were characterised as highly specific and "extra" reactions were not found with our cell panel. Inhibition (%): [1−(% rosettes after pretreatment with human AB Rh negative serum / % rosettes after pretreatment with test sera)] × 100. The percentage of rosettes after pretreatment with human AB Rh negative serum was not modified. ▲, F(ab′)$_2$ anti-4a (van Rood, 12123.2) which gives a titration curve similar to the whole serum = 4a$_1$ (not represented); ■, whole serum anti-4a (Claudia, Balner) = 4a$_2$; ○, whole serum anti-4b (van Rood, 9097.1) = 4b$_1$, ●, whole serum anti-4b (Hinchcliffe, Morris) = 4b$_2$

Table 1 Inhibition* of complement receptors by human anti-W4(4a) and anti-W6(4b) sera

Cell typing†	CRC %	CRC inhibition % (numbers in parentheses) after pretreatment with antisera anti — :						
		First series (HLA-A)	Second series (HLA-B)	Third series (HLA-C)	$4a_1$‡	$4a_2$	$4b_1$	$4b_2$
AA—V (1, 29/12, W17/4a, 4a)	19	A1(3), A29(0.)	B12(0.), BW17(−2)	—	(64)§	(60)	(3)	(0.)
BG (2, W26/12, W21/4a, 4a)	28	A2(0.), AW26(0)	B12(1.), BW21(5)	—	(74)§	(76)	(1)	(0.)
PH (1, 2/7, 8/4b, 4b)	26	A1(0), A2(0.)	B7(0.), B8(0.)	—	(1)	(10)	(65)	(67)
MW (2, X/W35, 8/4b, 4b)	22	A2(0.)	BW35(0), B8(10)	—	(6)	(8)	(68)	(72)
BT (2, 3/W35, W40/T2, T4/4b, 4b)	24	A2(0.), A3(0.)§	BW35(−0), BW40(6)	T2(0)§, T4(0.)	—	—	(50)	(79)
MH (1, X/8, W35/T4/4b, 4b)	18	A1(0)	B8(0.), BW35(0)	T4(0.)	—	—	(66)	(70)
LG (W26, 10.4/14, W35/4b, 4b)	22	AW26(0.), A10.4(3)	B14(0.), BW35(0.)	—	—	—	(70)	(70)
BW (W25, W32/W16, 18/T3/4a, 4b)	16	AW25(0.), AW32(0.)	BW16(0.), B18(0.)	T3(0.)	(1.)§	(1.)	(60)	(68)
CB (2, X/12, W15/T3, T4/4a, 4b)	26	A2(0.)	B12(0.), BW15(0.)	T3(0)	(30)§	(20)	(54)	(63)
BR (2, 11/5, W35/T1, T4/4a, 4b)	19.5	A2(5), A11(9)	B5(0.)§, BW35(6)	—	(77)§	(71)	(46)	(40)
JP (1, 3/7, W37/4a, 4b)	19	A1(0.), A3(0.)§	B7(0.), BW37(0.)	—	(31)	(30)	(78)	(82)
KH (3, 11/12, W40/4a, 4b)	21	A3(1)§, A11(—1)	B12(0.), BW40(1)	—	(0.5)	(0.)	(50)	(61)
Chronic lymphocytic leukaemia								
Hew. (2, X/W17, 15T1/4b, 4b)	60	A2(−2)	BW17(0.)	—	—	—	(94)	—
Dough, (1, 11/8, 12/4a, 4b)	65	A1(0.)	B8(0.)	—	(80)	—	(40)	—

All anti HLA antisera were used 1:2 dilution; anti-$4a_1$, $-4a_2$, $-4b_1$, $-4b_2$ as specified in Fig. 1. $2 \times 10^{\circ}$ VBS washed peripheral leukocytes were incubated 45 min at room temperature with 20 µl of the antisera in precipitin tubes. The cells were washed twice in VBS and sheep red blood cells covered by antibody and complement (EAC) were added (37 EAC: 1 lymphocyte ratio), spun down, incubated 8 min at room temperature, resuspended and the complement rosette percentage immediately counted. EA controls were 2–3% and E were negative throughout. Antisera were spun down at 14,000g for 5 min before use.

* Inhibition % as Fig. 1.

† July 1975 new WHO nomenclature for HLA system, except that T1 = HLA—CW1, T2 = HLA—CW2, T3 = HLA—CW3, T4 = HLA—CW4.

‡ See Fig. 1

§ $F(ab')_2$ fragments of HLA antisera were used at 1:2 dilution or according to Fig. 1 plateau dilution values in the case of $4a_1$. Whole sera gave substantially the same inhibition number, as they were also used in parallel.

same time for preincubating with BR cells, there was still only 73% inhibition (71% with anti-4a alone; 40% with anti-4b alone), indicating that one particular CR may be associated with both 4a, 4b products. These findings suggest that there may be different types of 4a/4b heterozygous gene products which might also be influenced by epistatic interaction with genes outside the MHS.

The inhibition of complement rosette formation was invariably partial: about 30% of rosettes were not inhibited. We believe this could be due to a specific reaction with lymphoid and not myeloid CR because the percentage fits with the number of non-lymphoid CRC in human peripheral blood[15] and the higher CR inhibition obtained when testing chronic lymphocytic leukaemia lymphocytes (Table 1). This conclusion is in agreement with our previous results in mice[16], where anti-H-2 sera inhibited only lymphoid complement receptors.

Only CR were specifically inhibited by anti-4a and anti-4b antisera. Sheep red blood cell (T) rosettes were not inhibited at all and Fc rosettes were nonspecifically inhibited by all the anti-HLA sera tested (Table 2) as anti-H–2 antisera behaved in the mouse[17]; nor did preincubation with rabbit anti human Ig affect CR numbers (unpublished).

Our results therefore suggest that the 4a, 4b gene products if not identical with CR may either constitute an integral part of them or be very closely associated with them. HLA–

Table 2 4a and 4b are not specifically associated with receptors other than complement receptors

Cell typing†	Anti-HLA sera used for inhibition (rosette inhibition (%))	
	Fc rosettes§	SRBC rosettes‖
AA-V	C1(27), ‡ A1(60), A29(67)*, B12(70), BW17(70), $4a_1$(69)*, $4a_2$(70).	C1(61), A1(0.), A29(−2), B12(0), BW17(1), $4a_1$(−2), $4a_2$(0)
BT	C1(28), A2(71), A3(70)*, BW35(72), BW40(73), T2(77)*, $4b_1$(74), $4b_2$(70).	C1(50) A2(0), A3(0.), BW35(0.), BW40(1), T2(0), $4b_1$(0), $4b_2$(0).
BR	C1(24), A2(71), A11(70), B5(71)*, BW35(70), T4(71), $4a_1$(60)*, $4b_1$(63)	C1(60). A2(0.), A11(0.), B5(0.) BW35(−2), T4(0.), $4a_1$(0.), $4b_1$(0).

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Values showed are mean of two different experiments.

* In these cases, $F(ab')_2$ fragments were also used.

† See Table 1.

‡ Underlined are the % rosettes in controls, see Fig 1.

§ 50 µl 1% ox cells in VBS covered by rabbit anti-ox IgG (provided by Dr M. B. Pepys) were mixed with 50 µl of 2×10^6 lymphocytes per ml in VBS in Precipitin tubes. They were spun down and counted straight away.

‖ 0.5 ml fresh 5% sheep red blood cells in VBS were mixed with 0 5 ml 5×10^6 per ml lymphocytes in VBS; prewarmed 5 min at 37 °C, spun down and incubated at 4 °C for a further hour. Rosettes were counted after careful resuspension.

A, –B and –C gene products are expressed in different molecules on the cell surface (that is they cap independently, see review in ref 18). Using these latter methods, we and others[19,20] have not been able to dissociate 4a/4b from HLA–B cell membrane gene products. Nevertheless, anti-4a and anti-HLA–B sera behave differently in our present experiments and in addition we found that 4a and 4b[21] have different linkage disequilibria with different HLA–B antigens in Negroid and Caucasoid populations, supporting the concept of separate genes controlling HLA–B and 4a/4b surface determinants. Furthermore, recent results of 50 diverse population studies of 4a, 4b have shown that they fit the Hardy–Weinberg equilibrium[12]. Also, interestingly enough, the gene frequencies (0.35 for 4a and 0.65 for 4b)[12] approximate to that for human C3 in North American Caucasoids ($C3^f$: 0 22 and $C3^s$: 0.77)[22]. $C3^f$ and $C3^s$ also have different binding capacities for complement receptors[23]. Our EAC (prepared with mouse complement) attach chiefly to the C3d receptor[24] of lymphocytes[24] and certain myeloid cells[25], and any attempt to relate the polymorphism of C3 to that of CR will have to take into account that we are probably detecting an inhibition of C3d receptors on lymphocytes. Although we have already shown that I region (I-C/S) products and CR are associated in the mouse[10,16], we are not yet in a position to make any direct comparisons with man but experiments designed to investigate the possible relationship between Ia antigens and 4a, 4b antisera activity are in progress Furthermore, the presence of anti-4a, 4b antibodies in pregnant women might affect (deleteriously?) foetal complement receptors.

In conclusion, these experiments strongly support the single-gene theory for the 4a, 4b system and also give an indication of a direct physiological role for an HLA antigen.

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Identification of a new oncofoetal antigen associated with several types of human carcinomas

IN experimental animals, oncofoetal antigens[1] have been found to be associated with both chemical-[2] and virus-induced tumours[3]. In man the two best known oncofoetal antigens are the α-foetoprotein (AFP) described by both Abelev[4] and Tatarinov[5] and the carcinoembryonic antigen (CEA) of the human digestive system identified by Gold and Freedman[6]. We describe here a different human oncofoetal antigen, common to several types of carcinomas and various foetal organs. This antigen has been identified by rabbit antisera raised against semipurified fractions of colon carcinoma soluble extracts. Because of its β-immunoelectrophoretic mobility, this antigen will be referred to as β-oncofoetal antigen (BOFA).

Primary tumours and adjacent normal tissues were obtained from surgical excisions, metastatic tumours from autopsies and foetal organs from spontaneous or therapeutic abortions. Soluble extracts of these tissues were prepared by a standard procedure: 0 2–0.5 cm³ fragments were suspended in two volumes phosphate buffer (0.03 M; *p*H 7.4) and homogenised in a Sorvall Omni mixer at 15,000 r p.m. for 30 min at 4 °C. After centrifugation of the homogenate at 30,000*g* for 30 min, the supernatant fluid was dialysed against 20 volumes deionised water for 24 h at 4 °C, and lyophilised. This extraction procedure yielded 40–50 mg dry powder per g wet tissue.

The extract from a large hepatic metastasis of a colon carcinoma was fractionated on Sephadex G-200. Each of four selected fractions was applied on a DEAE–cellulose column equilibrated with phosphate buffer (0.01 M; *p*H 8) and eluted stepwise with buffer of increasing molarity and decreasing *p*H. Twenty rabbits were immunised by repeated injections of 3 mg of the various DEAE–cellulose fractions in Freund's complete adjuvant. The three rabbits which received the colon carcinoma fraction eluted from Sephadex G-200 between the IgG and the serum albumin peak and then from DEAE–cellulose after the second buffer (phosphate 0 02 M; *p*H 7.4) produced the antisera which, after absorption with normal tissue extracts and plasma, became specific for BOFA. Every millilitre of antiserum was absorbed with 20 mg each of soluble extract from normal colon mucosa, normal liver, normal spleen, normal lung, AB Rh⁺ red cells and with 0.2 ml normal plasma.

In double diffusion the absorbed anti-BOFA antiserum revealed a single precipitin line with whole colon carcinoma extract but gave no lines with extracts of normal organs or with normal plasma (Fig. 1*a*). This antiserum gave a line of identity between extracts from carcinomas of the colon, lung, breast, liver, pancreas and from melanomas (Fig. 1*b*) as well as with extracts from the intestine, lung, liver and kidney of a human foetus of 16 week gestation (Fig. 1*c*). In all 34 carcinoma extracts (16 primary tumours and 18 hepatic metastases) and all 31 foetal organ extracts listed in Table 1 (with the exception of foetal skin), the same precipitin line was observed. In contrast, all 29 normal tissue extracts listed in Table 1, obtained from 14 different individuals, gave negative results, even at concentrations as high as 300 mg lyophilised powder ml^{-1}. Thus, at the level of sensitivity of the double diffusion test, BOFA seemed to be oncofoetal specific[7].

BOFA was also identified in extracts of human tumours (four colon carcinomas, four melanomas, two endometrial carcinomas) maintained by serial transplantation in nude mice[8–10] and in extracts of human tumour cell lines which had been established in tissue culture for several months or years. Controls using extracts of normal mouse tissue or

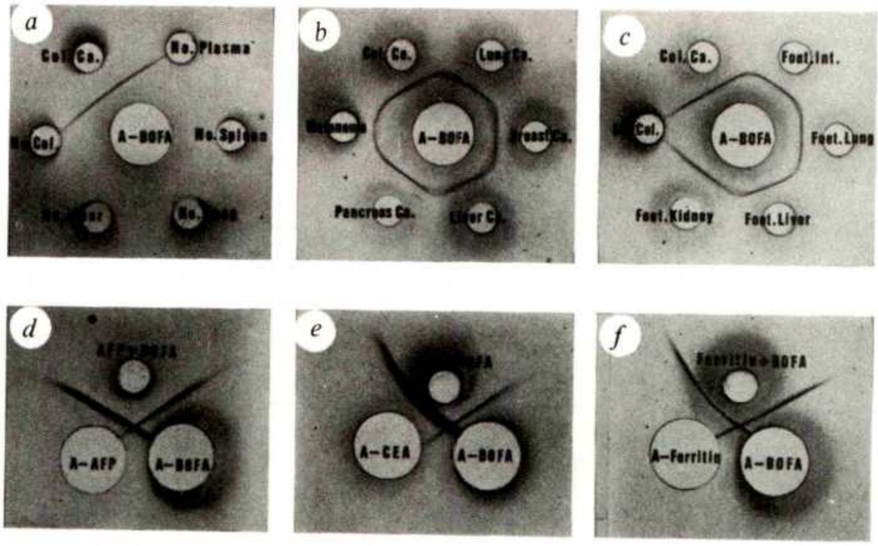

Fig. 1 Double diffusion in agar showing presence of BOFA in extracts of human tumoral and foetal tissues. *a*, *b*, *c*, Central wells: specific rabbit anti-BOFA serum absorbed with normal tissue extracts; antigens: *a*, colon carcinoma, normal neat plasma, normal spleen, normal lung, normal liver, normal colon; *b*, carcinoma of colon, lung, breast, liver, pancreas and melanoma; *c*, colon carcinoma, intestine, lung, liver and kidney of a foetus of 16 weeks' gestation, normal colon. All the antigens are whole lyophilised soluble extracts of tissues at a concentration of 100 mg ml^{-1}. *d*, Left well: specific anti-AFP serum (from Dr Sizaret, IARC Lyon France); right well: anti-BOFA serum; upper well: mixture of AFP and BOFA. *e*, Left well: specific anti-CEA serum (obtained as described previously[11]); right well: anti-BOFA serum: upper well: mixture of CEA and BOFA. *f*, Left well: specific anti-ferritin serum (from Behringwerke); right well: anti-BOFA serum; upper well: mixture of ferritin and BOFA.

concentrated culture medium containing foetal calf serum but no tumour cells gave no precipitin lines. These results indicate that BOFA is synthesised by the tumour cells.

BOFA is immunologically different from AFP, CEA or ferritin, as demonstrated by the crossed precipitin lines shown in Fig 1*d*–*f*. Ferritin was tested because antigens immunologically identical to ferritin, described as α-2H (ref. 12) or isoferritin[13,14], have been shown to possess oncofoetal properties. BOFA is also distinct from the normal glycoprotein (NGP) cross reacting with CEA[15] and the β-2-microglobulin[16]. Four other oncofoetal antigens described in the literature also seem to be different from BOFA. The γ-foetoprotein[17] and the leukaemia-associated antigen[18], unlike BOFA, were both detectable in foetal sera by the conventional double diffusion test. The antigen described by Klavins *et al.*[19] was detected in extracts of adult skin and was reported to have an isoelectric *p*H of 4.8 (ref. 20). The βS-foetoprotein was reported to be soluble in 0.6 M perchloric acid and to be eluted with the exclusion volume of Sephadex G-200 column. These properties differ from those of BOFA. Using immunoelectrophoresis, BOFA has been shown to have a β-2-mobility (Fig. 2*a*), and using isoelectric focusing on thin-layer polyacrylamide gel, a *p*H$_i$ ranging from 6 to 7. According to its elution on Sephadex G-200, BOFA has a molecular weight of 70–90,000. BOFA can be precipitated by 0.1 M perchloric acid and 33% saturated ammonium sulphate. Its antigenicity can be abolished by treatment with Pronase or by incubation at 70 °C for 30 min. In a caesium chloride gradient, the density of BOFA ranges between 1.30 and 1.32. These properties suggest that BOFA is a protein which does not contain a large amount of carbohydrate or lipid. Partial purification of BOFA was achieved by adsorption–elution from an immunoadsorbent

Table 1 Concentration of BOFA in various human tissues

Tissues	Tumour	Foetus	Normal
Colon and rectum	Primary: 165(80–320)* [8]† Metastatic: 303(83–483) [9]	88(75–120) [5]	4.0(2.5–5.0) [9]
Lung	Primary: 76(66–83) [2] Metastatic: 106(87–120) [3]	60(40–66) [5]	3.0(1.0–3.3) [4]
Liver	118(75–160) [3]	170(160–183) [5]	5.0(2.5–7.5) [4]
Kidney	47(42–50) [3]	140(83–160) [5]	4.1(3.3–5.0) [3]
Pancreas	80(66–91) [3]	83(73–93) [3]	NT
Breast	70(50–85) [3]	NT	2.6(2.0–3.6) [3]
Brain	NT	48(40–53) [5]	1.5(1.3–1.8) [3]
Skin	NT	18(15–27) [3]	1.3(1.0–1.5) [3]

*Mean and upper and lower limits in μg units per 100 mg lyophilised soluble extracts.
†Number of specimens tested.
NT, not tested.

Table 2 Detection of BOFA in various human cell lines

Origin	Cell line	Concentration of BOFA*	Surface immuno-fluorescence†
Colon carcinoma	HT29‡	50	++
	Col15§	66	+++
	Col25§	60	++
Breast carcinoma	SK-Br 3‡	60	++
	BT20‖	70	+++
Melanoma	SK-Mel 1‡	60	++
Endometrial carcinoma	END1§	66	++
Cervix carcinoma	Me-180‡	25	++
	HeLa¶	6	−
Burkitt lymphoma	Raji¶	3	−
Lymphoblastic leukaemia	Molt-4¶	0	−
Normal lymphocytes	Lik¶	0	−

*μg units per 100 mg lyophilised soluble extract.
†As determined by staining anti-BOFA antiserum treated living cells with fluoresceinated sheep anti-rabbit IgG conjugate.
‡Obtained from the Sloan–Kettering human tumour cell line bank, New York.
§Established by Dr S. Carrel in our Institute.
‖Obtained from Professor L. Ozzello. Institute of Pathology, Lausanne.
¶Obtained from the Swiss Institute for Experimental Cancer Research, Lausanne.

consisting of IgG from a specific rabbit anti-BOFA antiserum, coupled to Sepharose 4B. This method yielded small amounts of BOFA which were not sufficiently pure for use in a radioimmunoassay, but could be used as a preliminary reference antigen preparation for the quantitation of BOFA in different tissue and plasma samples.

As the conventional single radial diffusion test[22] was not sensitive enough to detect BOFA in patients' sera, we used the modification described by Rowe[23] in which the area of antigen–antibody precipitate is revealed by autoradiography after incubation of the plates with ^{125}I-labelled sheep IgG anti-rabbit IgG. By this method, which is about 30 times more sensitive than the conventional Mancini plates[22], it was possible to demonstrate the presence of BOFA in normal adult tissues as well as in the plasma of foetuses, of cancer patients and, at a slightly lower level, in the plasma of normal adults. The immunological identity between the small amount of BOFA present in various plasma samples with the reference BOFA from colon carcinoma extract was demonstrated by the radioactive double diffusion test developed by Abelev[24], using diluted reagents and a radiolabelled second antibody (Fig. 2*b*, *c*).

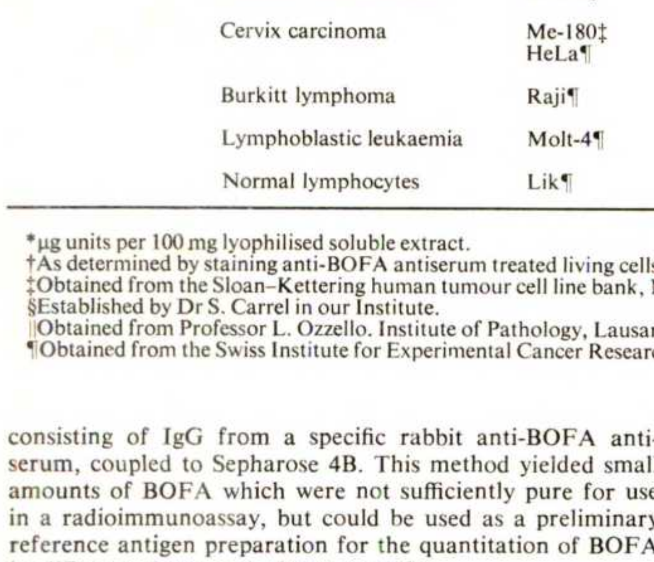

Fig. 2 *a*, Immunoelectrophoretic analysis of a whole colon carcinoma extract (Col. ca.) and a whole foetal liver extract (foet. liver). Antiserum: A-BOFA abs., rabbit anti-BOFA serum absorbed with normal tissues and plasma as described in the text. *b* and *c*, Autoradiographs of radioactive double diffusion in agar showing the immunological identity between BOFA present in colon carcinoma extracts (middle lower well, *b* and *c*) and the small amount of BOFA present in the plasma of a colon carcinoma patient (left well, *b*), a foetal plasma (left well, *c*) and a normal plasma pool (right well, *b* and *c*). Absorbed anti-BOFA antiserum was diluted 1/16, the colon carcinoma extract was at a concentration of 2.5 mg ml^{-1} (diluted 1/40 compared with Fig. 1*a*–*c*), whereas the three plasma samples are undiluted.

Results of the quantitative study of BOFA in various tissues, expressed in units of BOFA (corresponding to about 1 μg) per 100 mg lyophilised saline are shown in Table 1. The highest concentrations of BOFA were found in primary and metastatic carcinomas of the large bowel and in foetal liver and kidney. The concentration of BOFA was also relatively high in the 10 foetal placentae tested (mean, 101; range, 46–170), whereas in the placentae at the end of gestation it was only slightly lower (mean, 69; range, 40–80). In normal adult tissues the BOFA concentrations were 10–75 times lower than in the corresponding tumours. The most significant difference in BOFA concentration was observed by comparing the extract of a primary colon carcinoma, containing 320 units, with an extract of the normal colon mucosa, containing 2.5 units BOFA, both originating from the same patient.

In spite of the differences in tissue concentration, the plasma levels of BOFA in cancer patients and foetuses were not much higher than those in normal individuals. The quantitative study of BOFA in plasma samples from normal individuals and patients gave the following results (μg units ml^{-1}, mean and range): in 60 unselected blood bank donors, 1.0 (not detectable–3.4); in 34 patients with non-neoplastic inflammatory bowel diseases or liver cirrhosis, 2.5 (not detectable–5.1); among 57 patients with large bowel carcinomas, 18 with localised tumours (Dukes A and B), 5.8 (3.6–8.6); 22 with lymph node infiltration (Dukes C), 5.4 (3.8–8); 17 with distant metastases (Dukes D), 5.6 (4.0–7.2);

in 40 patients with various other types of carcinomas as well as melanomas and lymphomas, 5.4 (3.8–7.2). Elevated BOFA levels did not seem to be limited to a particular type of cancer. BOFA was measured before and after operation in the plasma of 16 patients who had a complete surgical resection of a large bowel carcinoma and whose CEA level dropped significantly or remained low after surgery[25]. In these patients the BOFA levels showed a moderate decrease 1–3 months after tumour removal. In 16 of these cases the preoperative values were 5.1 (3.8–6.6), whereas the lowest postoperative values were 1.9 (0.5–3.8). In cases of tumour recurrence[23], BOFA levels increased moderately in some, but not in all patients. It is too early to decide whether BOFA determination may have a clinical interest in connection with assays of other tumour-associated antigens such as CEA[25–28] and AFP[29].

The BOFA values were 6.3 (5.1–8.2) in the plasma of five foetuses of 14–20 week gestation, and remained relatively high at the end of gestation in 20 cord plasma samples tested: 8.2 (6.6–11) BOFA levels seemed to decrease progressively during the first 6 months of life, reaching values of 2.0 (not detectable–4.6) in the 30 plasmas of children aged 6 months–2 yr tested.

From the comparison between BOFA concentrations in tissues and in plasma, it seems that this antigen is not actively secreted by foetal or tumour cells. To determine the cellular localisation of BOFA, several human carcinoma or lymphoid cell lines were tested by indirect immunofluorescence. As shown in Table 2, BOFA could be demonstrated on the surface of eight of the nine carcinoma cell lines tested, whereas the fluorescence was negative on HeLa cells and on the three lymphoid cell lines, which contained little or no detectable antigen in their soluble extracts.

The functional role of this antigen remains to be determined. The relatively high concentration of BOFA in placenta and in cord serum at the end of gestation as well as its persistence in normal adult serum make it unlikely that it is immunogenic in cancer patients; on the other hand, one can hypothesise that BOFA may exert an immunosuppressive effect, protecting the foetus or the tumour cells from immunological rejection. This role has been attributed to several oncofoetal proteins, including α-2H (ref. 30), AFP[31] and the compound recently identified by Fauve *et al.*[32] in murine teratocarcinomas and in mouse trophoblast

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Corticosteroids inhibit prostaglandin production by rheumatoid synovia

PROSTAGLANDIN synthesis is inhibited by a wide variety of non-steroid anti-inflammatory drugs, and it has been suggested that the pharmacological effects of these drugs may be attributed to their inhibition of prostaglandin (PG) biosynthesis[1,2]. Although corticosteroids are among the most potent anti-inflammatory agents available, they lack inhibitory effects on PG synthesis in many systems[3–6]. We have investigated the effects of several anti-inflammatory drugs on PG production by rheumatoid synovial tissue *in vitro*. Non-steroidal agents inhibit PG synthesis by the synovia and their order of effectiveness is similar to that seen in other tissues[7]. Adrenocorticosteroids are also potent inhibitors of PG production by rheumatoid synovia, and the results of these experiments are reported here.

Explants of rheumatoid synovial tissue were prepared for culture from surgical specimens as described previously[8], and PG concentrations in the culture media were measured by radioimmunoassay. Rheumatoid synovial cultures produce mainly prostaglandins E_2 and $F_{2\alpha}$ (ref. 8), therefore the PGs were assayed by antisera to PGE_2 and $PGF_{2\alpha}$ and the concentrations were calculated in equivalents of PGE_2 and $PGF_{2\alpha}$, respectively. In one experiment, the PG metabolites, 15-keto-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ and 13,14-dihydro-15-keto-PGE_2 were measured using antisera of the desired specificities[9,10].

Table 1 Inhibition of PGE_2 production by rheumatoid synovial organ cultures by dexamethasone

Tissue R-66	Prostaglandin E_2 (μg ml^{-1})* Day 3†	 Day 6†
Control‡	6.80 ± 0.40	4.08 ± 0.50
Dexamethasone 10^{-4} M	0.27 ± 0.027	<0.12
Dexamethasone 10^{-6} M	$0 42 \pm 0 077$	<0.12
Dexamethasone 10^{-8} M	2.16 ± 0.31	0.133 ± 0.008
Dexamethasone 10^{-10} M	6.38 ± 0.26	4.13 ± 0.57

*PGE_2 concentrations were measured by radioimmunoassay, and each value is the mean of four determinations ±s.e.

†Medium from day 3 had been incubated with synovial tissue for the first 72 h of culture. After complete medium change, incubation was carried out for an additional 72 h and removed on day 6 for analysis.

‡Each group of four cultures was carried out in the same manner except for addition of dexamethasone at the concentrations indicated, or no additions to control cultures.

Table 2 Inhibition of PG production by rheumatoid synovial organ cultures by dexamethasone and hydrocortisone

Tissue R-67, Day 4	PGE_2	$PGF_{2\alpha}$	13,14-dihydro-15-keto- PGE_2	 $PGF_{2\alpha}$
		($\mu g\ ml^{-1}$)		
Control	8.5±1.1	1.65±0.29	1.94±0.33	0.264±0.022
Hydrocortisone 10^{-5} M	0.38±0.14	0.066±0.014	0.42±0.08	0.066±0.013
Dexamethasone 10^{-6} M	0.24±0.11	0.035±0.009	0.34±0.11	0.050±0.009

*0.009 $\mu g\ ml^{-1}$ of 15-keto-$PGF_{2\alpha}$ was present in the culture media of the control tissues and none could be found in the corticosteroid-treated cultures The 15-hydroxyprostaglandin dehydrogenase activity in the synovia therefore seems to limit the amount of 13,14-dihydro-15-keto compounds formed from Δ^{13}-reductase activity.

The production of PGE_2 and its inhibition by dexamethasone are shown in Table 1. Groups of four synovial cultures were incubated with four different concentrations of dexamethasone and compared with a control group without added drug. Concentrations of PGE_2 were reduced to $<6\%$ of the controls at both 10^{-4} and 10^{-6} M dexamethasone. Partial reduction of PGE_2 was seen at 10^{-8} M and there was no effect at 10^{-10} M. Slightly greater inhibition was observed in this and other experiments after the second incubation period on day 6 than on day 3, but the results were otherwise similar.

In another experiment, using a different synovial tissue, culture media were analysed for PGE_2, $PGF_{2\alpha}$ and three of their metabolites, the 13,14-dihydro-15-keto derivatives of PGE_2 and $PGF_{2\alpha}$ and the 15-keto derivative of $PGF_{2\alpha}$ (Table 2). In the presence of both hydrocortisone at 10^{-5} M and dexamethasone at 10^{-6} M, concentrations of PGE_2 and $PGF_{2\alpha}$ in culture media were reduced to $<5\%$ of untreated (control) cultures. Levels of the PG metabolites were similarly reduced, although to a somewhat lesser extent. The presence of these metabolites in culture media indicates significant activities of 15-hydroxyprostaglandin dehydrogenase and Δ^{13}-reductase in rheumatoid synovial tissue. The reduced concentrations of the PG metabolites is more consistent with an inhibitory effect of corticosteroids on PG synthesis than with an enhancement of their breakdown. The observation that PGE_2 and $PGF_{2\alpha}$ (and their respective metabolites) are reduced by similar factors suggests that corticosteroids inhibit PG synthesis at a stage before the formation of the endoperoxide intermediates, PGG_2 and PGH_2, since PGE_2 and $PGF_{2\alpha}$ are formed from the endoperoxide intermediates by separate pathways[11].

To investigate further whether the effect of corticosteroids on PG levels in synovial cultures is the result of either inhibition of synthesis or an enhancement of degradation, we examined the stability of PGE_2 in the experimental conditions. PGE_2 (1.0 $\mu g\ ml^{-1}$) was added to groups of cultures treated with dexamethasone and hydrocortisone at the beginning of each 72-h incubation period, and the recovery of PGE_2 was determined by comparison of PGE_2 concentrations in each pair of cultures (Table 3). The recovery ranged from 57% to 162% of PGE_2 added, which indicates that there is no significant degradation of PGE_2 in treated cultures. In a separate experiment, it was found that PGE_2 was also stable in untreated rheumatoid synovial cultures. These results show that the suppression of PGE_2 levels by corticosteroids cannot be accounted for by an increased rate of degradation in the culture media.

Table 3 Corticosteroid inhibition of PGE_2 production by rheumatoid synovia and the absence of increased PGE_2 degradation

Tissue R-70	Prostaglandin E_2 Day 3	($\mu g\ ml^{-1}$) Day 6
Control	5.4±0.67	1.98±0.29
Dexamethasone 10^{-6} M	0.19±0.05	0.008±0.002
Dexamethasone 10^{-6} M +PGE_2 1 $\mu g\ ml^{-1}$	1.2±0.10	0.58±0.12
Hydrocortisone 10^{-5} M	0.33±0.06	0.017±0.005
Hydrocortisone 10^{-5} M +PGE_2 1 $\mu g\ ml^{-1}$	1.95±0.11	1.21±0.23

See legend to Table 1 PGE_2 was added to one of two groups of dexamethasone- and hydrocortisone-treated cultures, to determine the stability of PGE_2 in these conditions

The data do not entirely exclude the possibility that corticosteroids could enhance PG degradation within synovial tissue, before the transport of PGs into culture media. This possibility seems unlikely, however, because of the reduced rather than increased levels of prostaglandin metabolites in cultures treated with corticosteroids.

Synovial tissue from the experiment reported in Table 1 was analysed for PGE_2 content at its conclusion. The results shown in Table 4 reveal that the PGE_2 content of the tissue is reduced by dexamethasone in parallel with the reduction in PGE_2 concentrations in the culture medium. The quantities of PGE_2 in untreated tissues were much lower than in culture media. For example, the culture media from the four control cultures contain over 32 μg PGE_2 at the end of the experiment on day 6 when the tissue was analysed (Table 1); in contrast, the pooled tissue from control cultures contained 0.111 μg, or $<1\%$ of that present in the medium. The results of the tissue analyses rule out the possibility that reduced PGE_2 concentrations in culture media produced by corticosteroids result solely from inhibition of transport out of the synovial tissue. It has been concluded by others[12] that corticosteroids may inhibit PG transport from adipose tissue.

Table 4 PGE_2 content of rheumatoid synovial tissue (R-66)

	μg PGE_2	Pooled tissue wet weight (mg)
Control	0.111	147
Dexamethasone 10^{-4} M	0.017	147
Dexamethasone 10^{-6} M	0.019	125
Dexamethasone 10^{-8} M	0.026	152
Dexamethasone 10^{-10} M	0.065	151

Synovial tissue from all cultures R-66 (see Table 1) were pooled from the four cultures comprising each group. The tissue was removed, rinsed and blotted dry and weighed at the conclusion of the experiment, after 6 d of incubation. The tissue was homogenised at 0 °C and the homogenate was extracted with diethyl ether after acidification to *p*H 3 (ref. 15).

Finally, in an experiment not shown here, prednisolone at 10^{-6} and 10^{-8} M, suppressed PGE_2 levels to 2% and 42% of control values respectively, after 72 h of incubation. Therefore similar inhibitory effects have been observed with hydrocortisone and two synthetic anti-inflammatory corticosteroids.

We conclude tentatively that corticosteroids inhibit the biosynthesis of PGs by rheumatoid synovial tissue, in the absence of any detectable increase in either degradation or storage of PGs in tissue explants. The mechanism of this inhibition remains unclear and is under investigation.

It is possible that corticosteroids inhibit PG production by a nonspecific toxic effect on cells in rheumatoid synovial tissue, but evidence against this is provided by histological sections of tissue from one experiment (Table 2) after 6 d in culture. Routine haematoxylin and eosin-stained preparations revealed no apparent differences between control and dexamethasone (10^{-6} M) treated tissues, and no histological evidence of cell necrosis was observed in either case.

The inability of corticosteroids to inhibit PG synthesis by various tissues, as intact cell preparations[4,5], tissue homogenates[3] or isolated microsomes[6], has been reported. This is in contrast to the sensitivity of PG synthesis by these same tissues and many others to inhibition by a variety of non-steroid anti-inflammatory drugs such as indomethacin and aspirin. In fact, we are unaware of any reports of inhibition of PG production by corticosteroids at the low concentrations used in our experiments and in those reported by Tashjian *et al.*[13]. Inhibition of PG synthesis by homogenates of human skin[14] and sheep seminal vesicles[15] by the steroid fluocinolone, has been reported, but in both cases concentrations of the drug exceeding 0.1 mM were used and resulted in only approximately 50% inhibition of PG synthesis. In addition, hydrocortisone had no effect on PG synthesis by the skin homogenates at a concentration of 0.28 mM[14]. Lewis and Piper have reported that corticosteroids inhibited PG-mediated vasodilatation in a perfused rabbit adipose tissue preparation[12], and they suggested that corticosteroids inhibited the transport of PG from the interior of adipose cells to the extracellular space. Hydrocortisone seemed to cause no significant change in PGE_2 content of adipose tissue, however, and it was not possible to measure PGE_2 directly by bioassay in effluent venous blood from either the control or treated preparations. Corticosteroids were shown to inhibit the ACTH-induced vasodilatation, which has been attributed to release of PGE_2 by adipose tissue, but Lewis and Piper presented no direct evidence that corticosteroids effected either PG synthesis or release.

In summary, corticosteroids have been shown to suppress the accumulation of PGE_2 and $PGF_{2\alpha}$ in culture media from explants of rheumatoid synovia and the evidence indicates that corticosteroids inhibit prostaglandin biosynthesis by this tissue.

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Hydrocortisone inhibits prostaglandin production by mouse fibrosarcoma cells

A VARIETY of non-steroid anti-inflammatory drugs are potent inhibitors of prostaglandin biosynthesis[1]. In fact, many of the clinically important anti-inflammatory actions of agents such as aspirin and indomethacin have been attributed to inhibition of prostaglandin synthetase[2]. Corticosteroids with anti-inflammatory activity, however, have not been regularly shown to be prostaglandin synthesis inhibitors[1,3,4] although reports of small degrees of inhibition have been made in broken cell preparations using very high concentrations (2.2×10^{-4}–4.4×10^{-4} M) of fluocinolone[5,6] or hydrocortisone[6]. Lewis and Piper have suggested, largely on the basis of indirect data, that some of the actions of corticosteroids in inflammation result from inhibition of the release, but not the synthesis, of prostaglandins[7]. We report here that hydrocortisone is a potent inhibitor of prostaglandin formation by a clonal strain of prostaglandin-producing cells in culture, and that the effect is not the result of an action on the prostaglandin release or transport mechanisms.

The cells used were the $HSDM_1C_1$ strain of mouse fibrosarcoma cells which synthesise and secrete substantial quantities of prostaglandin E_2 (PGE_2)[8,9]. Cell culture methods have been described[9] (see also legend to Fig. 1).

Addition of hydrocortisone to cultures of $HSDM_1C_1$ cells

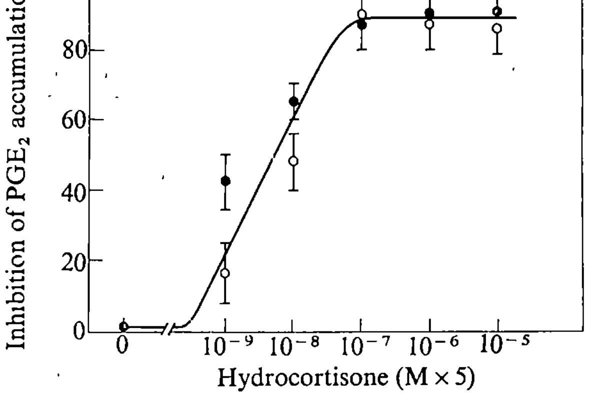


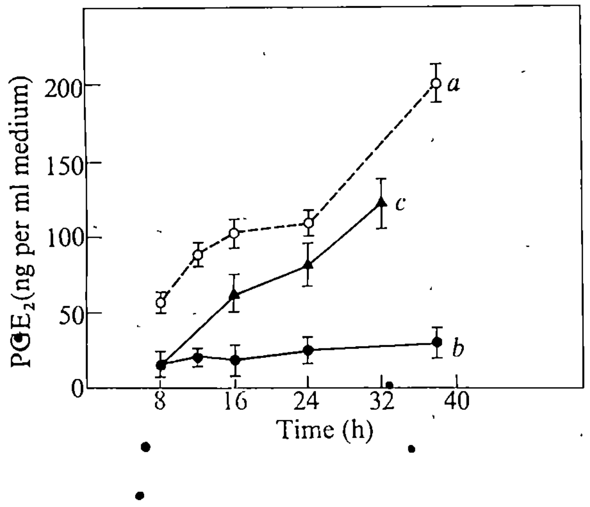
Fig. 1 Inhibition of PGE_2 accumulation in the medium of $HSDM_1C_1$ cultures produced by hydrocortisone. Cells were grown in Ham's F10 medium supplemented with 15% horse serum and 2.5% foetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Total cell protein was determined by the method of Lowry *et al.*[10] at the time of each medium collection. PGs[11,12] and PG metabolites[13,14] in unextracted medium were measured by serological techniques. We have shown that PGE_2 is the predominant prostaglandin synthesised and secreted by $HSDM_1C_1$ cells[8,9]. Media were usually assayed at final dilutions of 1/200 to 1/20,000, where there are no nonspecific effects of medium on the assay methods used. For measurements of intracellular PGs, the cells were homogenised in acidified (pH 3.5) Gey's balanced salt solution (about 2 ml mg^{-1} cell protein) containing indomethacin (1 μg ml^{-1}). PGs in the aqueous extract were then concentrated by extraction into diethyl ether as previously described[9]. Most experiments were performed using hydrocortisone sodium succinate (Solu-Cortef, Upjohn) although the same results were obtained with hydrocortisone. Each molar value read on the abscissa multiplied by 5 gives the final concentration of hydrocortisone in medium. Each point is the mean of duplicate culture dishes and the bars the ranges. Results are for culture periods of 4 (●) and 7 (○) d, respectively. After 4 d of incubation with or without hydrocortisone, medium was collected for assay and fresh control or hydrocortisone-containing medium was added for an additional 3-d period. In control cultures, PGE_2 accumulation averaged 150 ng ml^{-1} medium per 24 h.

produced a dose-dependent decrease in the accumulation of PGE_2 in the medium (Fig. 1). The half-maximum effect was observed at about 1×10^{-9} M hydrocortisone, and the extent of inhibition was 90–95% complete at maximum effective concentrations (5×10^{-7} to 5×10^{-6} M). In long term experiments (≥4 d), hydrocortisone concentrations of 10^{-7} M or lower had no significant effects on the rate of cell growth.

The time course and reversibility of the hydrocortisone effect are shown in Fig. 2. Inhibition of PGE_2 accumulation in medium was marked after 8 h incubation with the steroid hormone, and little or no further accumulation of PGE_2 occurred in the continued presence of 5×10^{-6} M hydrocortisone in spite of an increasing accumulation in controls. In cultures in which the drug was removed at the end of the 8-h treatment, there was a rapid return of PGE_2 accumulation in medium at a rate comparable with that in controls, indicating that the inhibition was not an irreversible toxic effect on the cells. Similar reversibility was observed in cultures treated with hydrocortisone for 24–72 h (data not shown). Other experiments showed that the inhibitory effect of the corticosteroid could be detected as early as 4 h after its addition to the culture medium.

Lewis and Piper stated that confirmation of their hypothesis of inhibition of prostaglandin release by corticosteroids might be accomplished by experiments with isolated cells[7]. We have, therefore, measured both the extracellular secreted PGE_2 and intracellular stored PGE_2 in control, hydrocortisone-, and indomethacin-treated cultures. If the steroid hormone were acting merely to inhibit the release of PGE_2, PG should accumulate intracellularly and exceed the amount found in control cells. Figure 3 shows that this is not what is found. Intracellular PGE_2 was not elevated in cells treated with hydrocortisone for 48 h although there was marked inhibition of PGE_2 accumulation extracellularly. Indomethacin, known to be a potent inhibitor of PGE_2 synthesis in $HSDM_1C_1$ cells[8,9,15], produced essentially complete inhibition of PGE_2 accumulation in medium and depressed intracellular PGE_2 to about 40% of control values (Fig. 3*a*). Removal of both hydrocortisone and indomethacin at 48 h was followed by a return of PG accumulation in the medium to control, or even elevated levels by an additional 24–48 h, again indicating that neither drug produced irreversible toxic effects on the cells.

Fig. 2 Time course and reversibility of the effect of hydrocortisone (5×10^{-6} M) on PGE_2 accumulation in the medium of $HSDM_1C_1$ cultures. Medium lacking (○) or containing hydrocortisone (● and ▲) was added at zero time to groups of replicate cultures. Duplicate dishes were taken and medium collected at each of the times shown for PG assay. *a*, Control cells without hydrocortisone; *b*, cultures received hydrocortisone continuously throughout; *c*, cultures received HC for the first 8 h only and control medium thereafter. There were no differences in total cell protein between groups throughout the experiment, averaging 0.80 mg per dish at zero time and 1.3 mg per dish at 38 h. Each symbol gives the mean value of duplicate dishes and the bars the ranges.



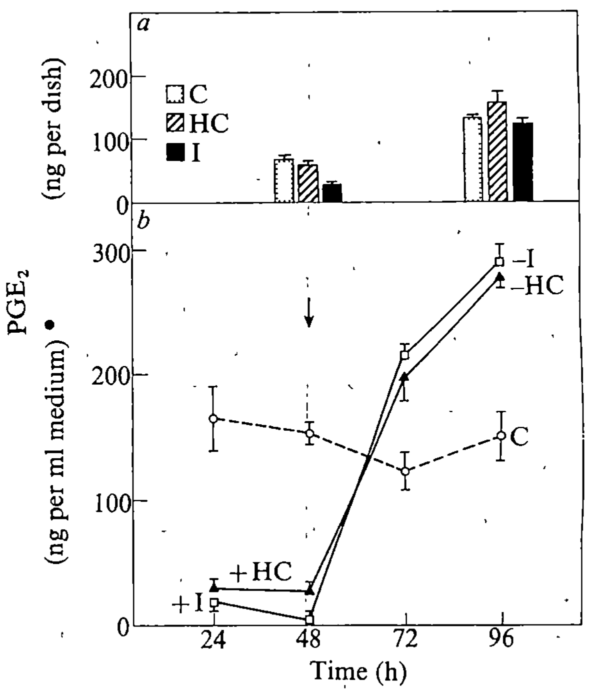


Fig. 3 Effects of hydrocortisone and indomethacin on intracellular (*a*) and extracellular (medium) (*b*) PGE_2 in $HSDM_1C_1$ cultures. Replicate dishes were cultured without (○) drug or with hydrocortisone (▲, 5×10^{-6} M) or indomethacin (□, 5.6×10^{-7} M) for 48 h. At that time (arrow), fresh medium lacking hydrocortisone or indomethacin was added to the previously treated dishes and incubations continued for an additional 48 h. Medium from all dishes was collected for assay and appropriate fresh medium added every 24 h. Each symbol gives the mean of duplicate dishes and the bars the ranges. *a*, Data for intracellular PGE_2 in control (C) and hydrocortisone (HC)- and indomethacin (I)-treated cultures at 48 and 96 h Each block represents the mean of duplicate dishes and the bars the ranges. In three additional experiments intracellular PGE_2 content in HC-treated cultures was 74, 82 and 68% of control.

We conclude that in the $HSDM_1C_1$ cell culture system hydrocortisone is a potent inhibitor of the accumulation of PGE_2 in the extracellular medium. The effect cannot be explained by a simple inhibition of PGE_2 release or transport[7] because there is no concomitant increase in intracellular PGE_2, in fact, a decrease in intracellular PGE_2 was usually observed. We do not believe that hydrocortisone has a nonspecific, toxic effect on cell metabolism because its actions occur at low steroid concentration, are reversible, occur rapidly, and are not accompanied by a decrease in the growth rate of the cells. It could be argued that hydrocortisone acts to cause the observed effect by accelerating the degradation of PGE_2 or by altering the ratios of PGE_2 to $PGF_{1\alpha}$ or $PGF_{2\alpha}$. We do not believe that enhanced breakdown of PGE_2 is the explanation, for we cannot detect the metabolites 13,14-dihydro-15-keto-PGE_2, 13,14-dihydro-15-keto-$PGF_{2\alpha}$ or 15-keto-$PGF_{2\alpha}$ in hydrocortisone-treated cultures (0.5 ng ml^{-1} of these metabolites could have been measured in control and hydrocortisone-treated culture media by our radioimmunoassay techniques). In addition, exogenous PGE_2 is not degraded by cells previously treated with hydrocortisone. PGE_2 (51 ng ml^{-1}) was added to cultures treated with hydrocortisone (5×10^{-6} M) for 24 h. Control hydrocortisone-treated cultures received no added PGE_2. In a subsequent 5-h incubation (from 24–29 h), 99±3% (mean ± s.e.) of the added PGE_2 was recovered. When culture medium was assayed for $PGF_{2\alpha}$ as well as PGE_2, no changes in the ratios of PGE_2 to $PGF_{2\alpha}$ were found; the ratios of PGE_2 to $PGF_{2\alpha}$ in one experiment were 31 and 26 in control and hydrocortisone-treated cultures respectively, and in a second experiment, 34 and 32, respectively.

We propose therefore that in intact $HSDM_1C_1$ cells, hydrocortisone inhibits PGE_2 accumulation in medium by interfering with PG synthesis. Although the locus of this effect remains unknown at present, the unaltered ratios of PGE_2 to $PGF_{2\alpha}$ indicate that the block in synthesis precedes endoperoxide formation. The simplest explanation at the moment is that the corticosteroid binds to membrane structures and prevents activation of the prostaglandin synthesis system of the intact cell. The similarity of our findings to those of Kantrowitz *et al.*[16] in a different experimental system, lead us to believe that these effects may be a more general action of corticosteroids and may be important in their activity as anti-inflammatory agents.

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Spontaneous calcium action potentials in a clonal pituitary cell line and their relationship to prolactin secretion

IT has been suggested that in the adrenal medulla calcium ions enter the cell as a result of depolarisation caused by acetylcholine[1]. During this process it is conceivable that a mechanism of membrane potential-dependent calcium permeability change, that is, the calcium action potential, is involved. A certain amount of calcium ions may enter the cell during an action potential and the modulation of the action potential frequency may provide a sensitive way to control the calcium entry. Similar mechanisms may be involved in other secretory systems. Here I describe an attempt to prove this hypothesis in anterior pituitary cells.

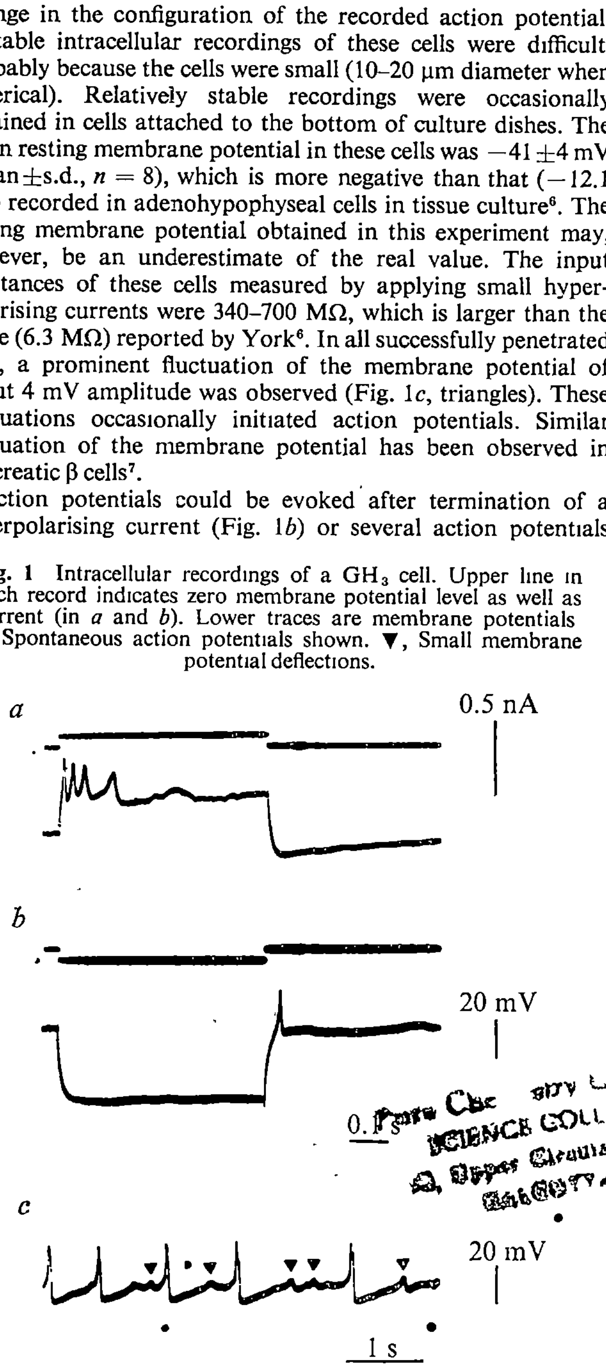
A clonal cell line (GH_3) isolated from a rat anterior pituitary tumour has been shown to secrete continuously both growth hormone and prolactin[2,3]. A hypothalamic releasing factor (thyrotropin releasing factor TRF) stimulates prolactin secretion and inhibits growth hormone secretion at nanomolar concentrations[4].

Electrical membrane properties of these cells were studied using intra-and extracellular microelectrode recording techniques, and their correlation with secretory activities is discussed. Cells were grown in Ham's F10 medium supplemented with 15% horse serum and 2.5% foetal calf serum in an atmosphere of 5% CO_2 in air. Intracellular recording and current injection using a bridge circuit were carried out as described previously[5] in a saline of the following composition: NaCl 142.6 mM, KCl 5.6 mM, $CaCl_2$ 10 mM buffered by 5 mM HEPES–NaOH to *p*H 7.4. A high calcium concentration in this saline seemed to enable good microelectrode penetration of the cell. A suction microelectrode with an inner tip diameter of less than 5 µm was used for the extracellular recording. A small part of the cell surface membrane was slowly sucked into the electrode (about 5–10 µm) making a good electrical contact. The action potentials recorded in this way usually have a positive–negative deflection of 0.1–1 mV peak-to-peak amplitude (Fig. 2*a*). For extracellular recordings a saline of the following composition was used: NaCl 152 mM, KCl 5.6 mM, $CaCl_2$ 1.8 mM, sucrose 38 mM, buffered by 5 mM HEPES–NaOH to *p*H 7.4. Sucrose was added to adjust the tonicity of a saline to that of culture medium. All experiments were carried out at room temperature (19–26 °C). The frequency of spontaneous action potentials is affected by the mechanical deformation caused by the suction electrode. To avoid this artefact as much as possible, action potential frequency was estimated only after the spontaneous firing rate had been stable for more than 5 min with little or no change in the configuration of the recorded action potential.

Stable intracellular recordings of these cells were difficult, probably because the cells were small (10–20 µm diameter when spherical). Relatively stable recordings were occasionally obtained in cells attached to the bottom of culture dishes. The mean resting membrane potential in these cells was -41 ± 4 mV (mean±s.d., $n = 8$), which is more negative than that (-12.1 mV) recorded in adenohypophyseal cells in tissue culture[6]. The resting membrane potential obtained in this experiment may, however, be an underestimate of the real value. The input resistances of these cells measured by applying small hyperpolarising currents were 340–700 MΩ, which is larger than the value (6.3 MΩ) reported by York[6]. In all successfully penetrated cells, a prominent fluctuation of the membrane potential of about 4 mV amplitude was observed (Fig. 1*c*, triangles). These fluctuations occasionally initiated action potentials. Similar fluctuation of the membrane potential has been observed in pancreatic β cells[7].

Action potentials could be evoked after termination of a hyperpolarising current (Fig. 1*b*) or several action potentials

Fig. 1 Intracellular recordings of a GH_3 cell. Upper line in each record indicates zero membrane potential level as well as current (in *a* and *b*). Lower traces are membrane potentials *c*, Spontaneous action potentials shown. ▼, Small membrane potential deflections.



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were observed with each depolarising pulse (Fig. 1*a*). The action potentials were followed by an after-spike hyperpolarisation, and had several characteristics in common with a calcium action potential[8]. Thus they were not blocked by tetrodotoxin (2×10^{-6}M) nor by substituting equiosmolar Tris-HCl for the NaCl and HEPES–NaOH, but could be abolished by adding $LaCl_3$ (1 mM) or $MnCl_2$ (5 mM) to the medium. Therefore it is likely that these action potentials were due to a membrane conductance increase to calcium ions.

Spontaneous action potentials were recorded extracellularly. All cells showed spontaneous action potentials at random intervals with a frequency of 52 ± 20 min^{-1} ($n = 20$) at room temperature (Fig. 2*b*). The frequency of action potentials in any given cell was stable (coefficient of variation: 15 in average of 6 cells) although the frequency varied from cell to cell (coefficient of variation: 38). After TRF acetate was added to the medium, action potential frequency was measured in randomly chosen cells. The mean frequency in the presence of 30 nM TRF was 91 ± 32 min^{-1} ($n = 13$) which is significantly larger than the control ($P < 0.001$), whereas with 1 nM TRF the mean frequency was 52 ± 26 min^{-1} ($n = 6$) which is not different from the control. Moreover, the spike frequency after addition of 231 nM [πMeHis^2]–TRF (inactive TRF derivative)[4,9] was 60 ± 26 min^{-1} ($n = 9$), which is not different from the control.

In this system[10,11] as well as in many others[12] extracellular calcium ions have been shown to be required for hormone secretion. The level of prolactin secretion of the anterior pituitary cells used in this experiment is reported to increase immediately after addition of TRF (0.3 μM) to several times the basal level[4]. Similarly the frequency of spontaneous calcium action potentials increased to about two times the control after adding a small amount of TRF. [πMeHis^2]–TRF did not affect the action potential frequency or the hormone secretion[4,9].

The correlation between hormone secretion and calcium action potentials in this system in various conditions suggests that calcium ions traversing the membrane during action potentials may be related to the hormone secretion, as has been suggested in pancreatic β cells[13].

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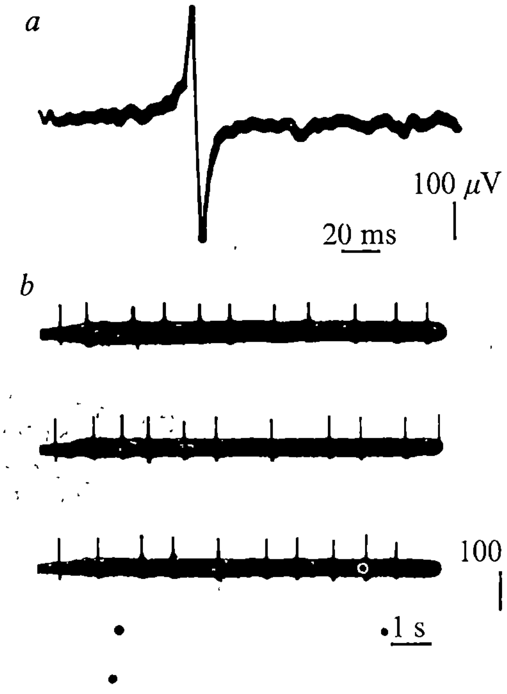
Fig. 2 Extracellular recordings of spontaneous action potentials. Upward deflection indicates positive potential change at the electrode referring to the bath. *a*, Fast sweep record; *b*, spontaneous action potentials recorded with slower sweep speed. *a* and *b* are records from different cells.



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Effect of oestrogen antagonist on hypothalamic oestrogen receptors

THE oestrogen antagonist CI-628 can block completely oestrogen-induced sexual receptivity in rats even when the antagonist is given as long as 6 h after oestrogen[1]. But as CI-628 does not displace labelled oestradiol from hypothalamic tissue when given at times when it does block the behavioural effects of the oestrogen, the inhibition is not due to simple competitive inhibition. The behavioural inhibition, however, might be due to an inhibition of the action of oestrogen on hypothalamic receptors. This was suggested by the report that oestrogen antagonists can deplete cytoplasmic oestrogen receptors in the uterus[2], but fail to stimulate production of new receptor, a process which is induced by oestrogen. We have now shown that CI-628 depletes hypothalamic oestrogen receptors, that receptor replenishment occurs very slowly and that CI-628 delays receptor replenishment after oestradiol treatment, suggesting that receptor replenishment might be important for the behavioural effects of oestradiol.

Eighty female rats were ovariectomised at 8 weeks of age and used 4–25 d later Groups of five rats were injected intravenously with 2 5 μg of oestradiol, 500 μg of CI-628 (1-[2-(*p*-[α-(*p*-methoxyphenyl)-β-nitrostyryl] phenoxy) ethyl] pyrrolidine monocitrate) (Parke-Davis) or with oestradiol followed by CI-628, control animals were not injected. The animals were killed 1–24 h after injection. The hypothalamus was removed, homogenised in TE buffer (10 mM Tris, 1 5 mM EDTA, 1 mM dithiothreitol, *p*H 7.4) and centrifuged for 1 h at 105,000*g* to yield a high speed supernatant (cytosol) fraction Twenty-five microlitres of 2,4,6,7-^{3}H-oestradiol (specific activity 104 8 Ci mmol^{-1}, New England Nuclear) in TE buffer was added to 0 1 ml of each cytosol to give a concentration of 1×10^{-9} M Further 0.1-ml aliquots were treated with ^{3}H-oestradiol plus a 100-fold excess of unlabelled oestradiol. The samples were incubated overnight at 4 °C. A third aliquot was taken for protein determination using the Miller modification[3] of the Lowry procedure Protein concentrations were between 8 and 10 mg ml^{-1} Cytoplasmic oestradiol binding was assayed using a Sephadex G-25 minicolumn technique[4]. Specific binding was determined from the difference in binding between the cytosols incubated with ^{3}H-oestradiol and cytosols treated with ^{3}H-oestradiol plus a 100-fold excess of unlabelled oestradiol. Binding was determined as d.p.m. per mg protein and is expressed as percentage of control. The control receptor levels varied in different experiments with a mean of 552 4 d p.m per mg protein This technique

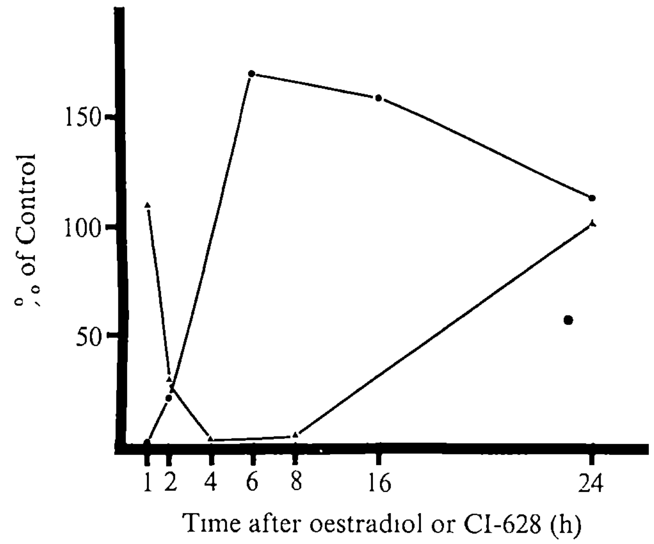

Fig. 1 Relative levels of saturable binding in cytoplasm from hypothalamic tissue of ovariectomised rats at various times after intravenous treatment with oestradiol (●) or the anti-oestrogen CI-628 (▲)

measures only unfilled receptor sites and thus may give an underestimate of total sites.

Figure 1 shows the changes which occur in the level of hypothalamic oestrogen receptors after intravenous treatment with either oestradiol or CI-628. One hour after oestradiol treatment the receptors were completely depleted, partial replenishment was seen after 2 h, and at 6 h receptor levels were more than 150% of control values. Levels returned to control values by 24 h after oestrogen treatment The pattern of change after CI-628 treatment was different from that produced by oestrogen. Receptor depletion was slower, reaching a minimum value 4 h after treatment; replenishment was slower and, for the dose used and at the times of measurement, no overshoot was observed Others have reported that CI-628 induced receptor depletion, but found no receptor replenishment[5] as observed in the study reported here.

Table 1 shows the effects of CI-628 administered at various times after oestradiol treatment on receptor levels 24 h after oestrogen had been given Receptor levels were quite low in each case. Comparison of these data with those in Fig 1 suggests that CI-628 prevents, at least at the times examined, replenishment of receptors when given shortly after oestradiol and depletes receptors which are generated by long term (8 or 16 h) oestrogen stimulation.

Our data suggest that the inhibitory effects of CI-628 on sexual activity are due to an inhibition of receptor replenishment. But the action of CI-628 may be more complex. It has been shown that the oestradiol can be translocated to the nucleus of uterine cells after treatment with anti-oestrogen but that it has little effect on uterine growth[6]. Thus anti-oestrogens can both block cytoplasmic receptor replenishment and interfere with interactions of oestrogen-receptor complexes with the nucleus.

Table 1 Effect of CI-628 on hypothalamic cytoplasmic oestrogen receptor levels

Hours after E2 when CI-628 was given	% of Control
2	25.2
4	20.4
8	1.6
16	7.5

Animals were killed 24 h after treatment with oestradiol.

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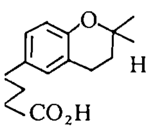
New antisickling agent 3,4-dihydro-2,2-dimethyl-2H-1 benzopyran-6-butyric acid

RECENT efforts to develop therapeutic agents for the treatment of sickle cell disease have led to the discovery of several antisickling agents[1]. We now report a new such agent, 3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-butyric acid (DBA) (Compound 1). This compound efficiently prevents and reverses sickling, and has no acute toxicity in male mice.

1



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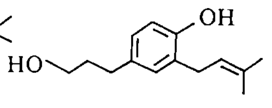

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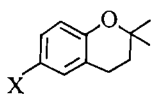


a, X=H
b, $X=CO(CH_2)_2CO_2H$

Fagara xanthoxyloides Lam, a small tree with shining aromatic leaves is common in coastal areas of West Africa. The roots, which have a strong spicy taste, are used in Nigeria as chewing sticks which are chewed to a brush and used to clean the teeth. Various natural products[2,3] have been isolated from the plant, and the crude aqueous extract of the roots has been investigated for its antimicrobial[4] and antisickling[5] activity. Root bark and root wood extracts gave, in addition to other natural products (unpublished results of J.I.O , D.E.U E. and V.U.E.), xanthoxylol (compound 2). In preliminary bioassays on xanthoxylol (carried out by Dr A. Isaacs-Sodeye, University College Hospital, Ibadan) it seemed to possess antisickling activity. We have chemically modified xanthoxylol to DBA, and have found that the latter is a very active antisickling agent. It has also been synthesised efficiently, in 90% overall yield, by Friedel Crafts acylation of 2,2-dimethylchroman (3*a*) (obtained from dihydrocoumarin) with succinic anhydride, followed by Clemmensen reduction of the keto-acid (3*b*). ^{14}C-DBA was prepared using 1,4-^{14}C-succinic anhydride in the acylation step

Sickling was produced by one of the following methods: (1) reduction with 2% metabisulphite; (2) evacuation at 30 mmHg with a water aspirator for 10 min at 37 °C; and (3) bubbling a mixture of 95% N_2 and 5% CO_2 through a saline suspension of sickle cells for periods of 10 min to 2 h at 37 °C. In all cases about 90% sickling was attained.

Hanging drop preparations (10 µl) of deoxygenated cell suspension were maintained in the deoxygenated state and treated with various amounts of DBA. One thousand cells were counted at suitable intervals by light microscopy (Zeiss, ×600). As Table 1 shows, the effect of DBA was already apparent after 30 min, the minimum effective concentration being 0.9 mM. In addition, the cell suspensions were placed in 10-ml Erlenmeyer flasks arranged in series in a 37 °C waterbath, and

Table 1 Effect of DBA on deoxygenated sickle cells at different times

Concentration (mM)	30 min % Sickle cells	30 min % Varied shape cells	30 min % Normal shape cells	60 min % Sickle cells	60 min % Varied shape cells	60 min % Normal shape cells	90 min % Sickle cells	90 min % Varied shape cells	90 min % Normal shape cells
Control	69	29	2	69	29	2	72	26	2
10	1	7	92	0	11	89	3	3	94
6	2	18	80	2	21	77	2	12	86
3	3	36	61	4	25	71	6	34	51
1.8	8	31	61	3	41	56	7	40	53
0.9	46	32	22	44	46	10	23	54	23

1,000 cells were counted.

deoxygenated. Various concentrations of the drug were added, the flasks were incubated for 1 h and the cells were fixed by dilution with deoxygenated, buffered formalin. The results obtained on these fixed preparations were similar to those shown in Table 1. The erythrocyte indices were not affected when sickle cells were incubated with DBA and deoxygenated.

The inhibitory effect of DBA on sickle cell formation was tested by incubating sickle cell suspensions with three concentrations of DBA for 1 h, deoxygenating the cells as described previously, and then counting the percentages of sickle and normal cells. The control sample remained oxygenated, and contained 98% (patient *A*) and 78% (patient *B*) normal shaped cells, respectively. As Table 2 shows, the antisickling effect was already observed with 1 mM DBA, and inhibition of sickling was almost complete at 13 mM. The antisickling effect with 7.5 mM DBA is shown in Fig. 1.

As Fig. 2 shows, the oxygen pressure (in mmHg) at which the test blood was half-saturated (P_{50}) did not change significantly from the control specimen, a slight shift, if any, occurring towards the right. This again is unlike most other antisickling agents, for example, cyanate[8], dimethyl adipimidate[10] and nitrogen mustard[9], which shift the oxygen affinity curve to the left. These results indicate that there may be no interference with the delivery of oxygen to tissues.

When sickle cell anaemia haemolysates were exposed to different concentrations of DBA for 1 h gelling was inhibited at a concentration of 2 mM (Table 3).

^{14}C-DBA incorporation studies (Table 4) showed that very little of the drug used for incubation actually enters the red cells. Of the counts incorporated most were associated with non-haemoglobin protein (trichloroacetic acid (TCA) soluble part). Carboxymethyl cellulose chromatography, at *p*H 6.7, of globin from the lysate of cells incubated with ^{14}C-DBA revealed that the ^{14}C counts remained just above background and were distributed nonspecifically throughout the column. The ^{14}C counts were associated with neither α nor β chains. The α/β ratio as estimated from ^{3}H counts was 1. Thus unlike other antisickling agents such as potassium cyanate which binds to the α and β chains of oxyhaemoglobin[15,16], DBA does not bind covalently to sickle haemoglobin. The activity of DBA thus seems to be due to non-covalent binding to haemoglobin or interaction with the red cell membrane.

The rate of potassium loss from sickle cells provides an accurate method of measuring the degree of sickling[17] In a preliminary experiment, a sample of sickle blood (Hct 30%) was incubated for 30 min at 37 °C in air. Ouabain (10^{-4} M) was then added to inhibit active cation transport. The sample was then deoxygenated (95% N_2, 5% CO_2) for 30 min. Another sample of sickle blood containing 7 mM DBA was submitted to the same treatment. The content of potassium in the plasma was determined by flame photometry (Instrument Lab. 243). This showed that red cell potassium loss induced by hypoxia was decreased by 6 meq l^{-1} of cells in the treated sample with DBA. Experiments to observe the effect of the drug on red cell potassium loss using physiological gas mixtures for deoxygenation are in progress.

Table 2 Antisickling effect of DBA

	DBA (mmol)	% Normal cells
Patient *A*	3 8	87
	8.0	93
	13.0	95
Patient *B*	1.0	48
	6.0	64

Table 3 Inhibition of gelation by DBA

DBA concentration (mol × 10^{-4})	Hb concentration g per 100 ml
Control (without DBA)	29 7
1.98	35.0
3.92	35.7
9.52	37.6 (non-gelling Hb conc.)

A unit of blood was obtained after exchange transfusion therapy of a patient whose starch gel electrophoresis revealed HbA = 51 9 and HbS = 48.1. The haemolysate was prepared by the method of Drabkin[11], and was concentrated by pressure dialysis and ultrafiltration against 0.15 M potassium phosphate at *p*H 7.35. The lysate (0.5 ml, with and without DBA) was deoxygenated in a 10-ml Erlenmeyer flask at 25 °C on a rotary shaker under a stream of water-equilibrated N_2. Gelling inhibition studies were carried out according to the method of Bookchin *et al.*[12].

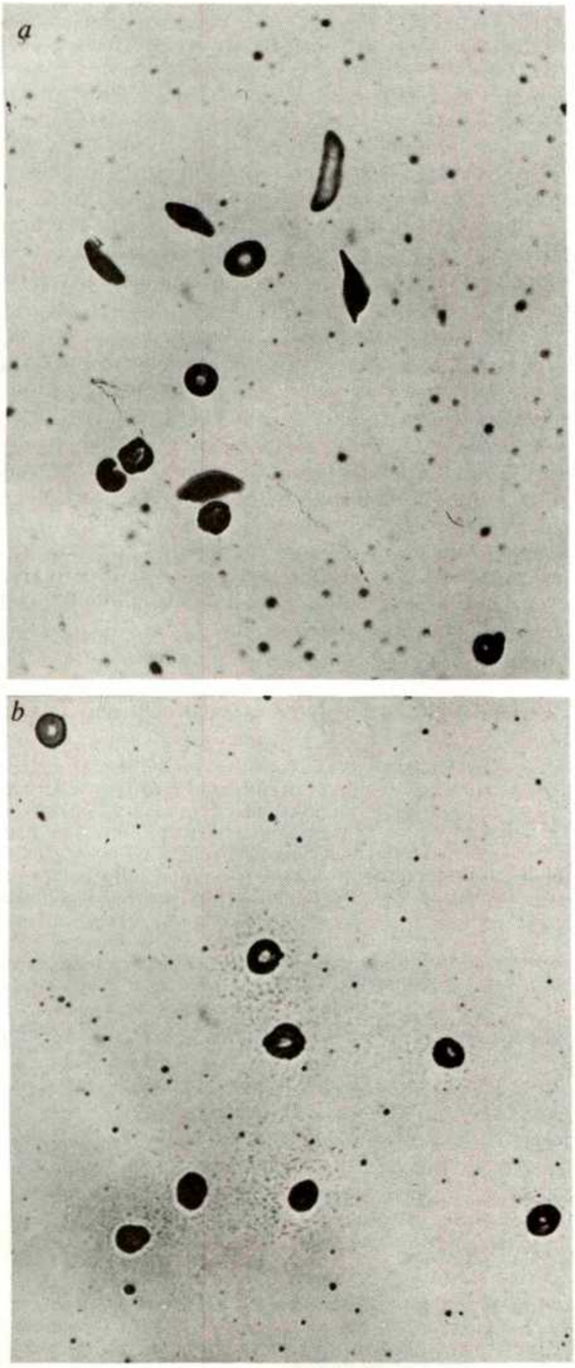
Table 4 Incubation of sickle cells with ^{14}C-DBA

Fraction	^{14}C c p.m
(*a*) Total c.p.m.	about 2.40 × 10^6
(*b*) Clear supernatant	2 34 × 10^6
(*c*) Stroma	1,400
(*d*) Haemolysate, hot TCA insoluble	1,600
(*e*) TCA filtrate	25,200

Packed sickle cells (0.5 ml) were washed three times with isotonic saline and incubated at 37 °C for 1 h with *p*H 7 4 buffer, phosphate buffered-saline (PBS), or Krebs Ringer bicarbonate (KRB), and ^{14}C-DBA 1.09 × 10^5 d.p.m. μmol^{-1} (11 mM solution, total count about 2.40 × 10^6 c p.m. (fraction *a*)). ^{3}H-leucine was used as an internal control to measure incorporation into globin After incubation, the packed cells were separated from the clear supernatant buffer by centrifugation at 2,000 r.p m for 10 min, and the radioactivity of the supernatant was counted. The cells were then washed twice with isotonic saline until the counts in the washing were negligible, and the radioactivity of the original supernatant and combined washings was obtained (fraction *b*) The cells were lysed with four volumes of water, and the stroma was removed by centrifugation at 15,000 r p.m for 20 min at 4°C The stroma was washed several times with a large excess of 1 mM EDTA The ^{14}C counts of the residual stroma was 1,400 c.p m. (fraction *c*). Sample of lysate was then fractionated with a 0 45-μm millipore filter into hot TCA-soluble (non-haemoglobin) and hot TCA-insoluble (haemoglobin) protein fractions, the countings of the two fractions showed that the radioactivities of the haemoglobin protein and non-haemoglobin protein were, respectively, 1,600 and 25,200 c p.m. (fractions *d* and *e*) Globin was precipitated from the remainder of the haemolysate by extraction with hydrochloric acid-acetone, and further purified by carboxymethyl cellulose chromatography using a modification of the method originally described by Clegg *et al.*[13] and detailed elsewhere[14]. Samples were counted using a Packard Tricarb liquid scintillation counter. No significant ^{14}C counts accompanied the α and β haemoglobin chains.

The acute toxicity of DBA was studied (at the Teijin Institute for Biomedical Research, Tokyo) by both intraperitoneal and oral administration to male mice. Intraperitoneal DBA caused sedation at 250 mg kg^{-1}; sedation, ataxia, analgesia and myorelaxation at 500 mg kg^{-1}; convulsion and other toxic syndromes at 750 mg kg^{-1}, and Straub's tails, chronic convulsions, depression of respiratory rate and death at 1,000 mg kg^{-1}. The LD$_{50}$ was judged to be 710 mg kg^{-1}. Oral administration of DBA up to 3,000 mg kg^{-1} resulted in weak sedation; with doses greater than 1,000 mg kg^{-1}, we also observed piloerection and weak myorelaxation. The LD$_{50}$ for oral administration was thus judged to be > 3,000 mg kg^{-1}.

Fig. 1 Antisickling effect of 7.5 mM DBA (Zeiss, ×600). Fresh heparinised blood samples collected from patients with homozygous sickle cell anaemia were kept at 4 °C, and used within 6 h of venipuncture. The erythrocytes were washed three times with isotonic saline. A cell suspension was made by diluting the cells tenfold with phosphate-buffered saline at *p*H 7.2. *a*, Control cell suspension was incubated at 37 °C in buffer for 1 h then deoxygenated for 1.5 h by method (*c*) (see text). *b*, Cell suspension with added 9 mM DBA was incubated similarly and deoxygenated. Cells treated with DBA had no sickle forms.



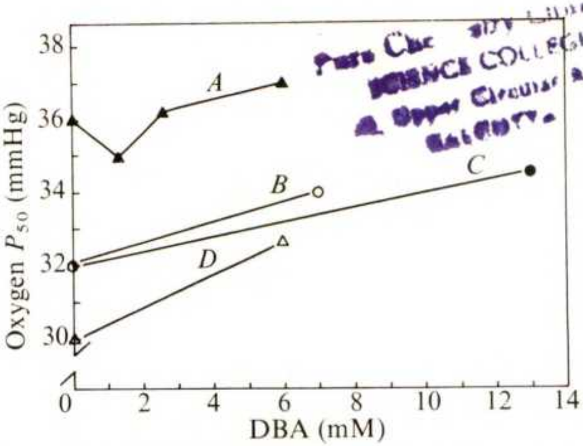


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Fig. 2 Effect of DBA on the oxygen affinity of HbS. The P_{50} values from four patients (*A*–*D*) with sickle cell anaemia were determined before and after treatment with various concentrations of DBA. The P_{50} value of blood incubated with DBA was determined by the mixing technique of Edwards and Martin[6]. Whole blood (5 ml) or cell suspensions were used. The test samples were incubated with different concentrations of DBA for 1 h. Oxygen saturation was measured in a co-oximeter model 182 (Instrumentation Laboratory, Inc.). Measurements of P_{O_2}, P_{CO_2} and *p*H were made with a model 213 blood gas analyser (Instrumentation Laboratory, Inc.). For calculation of P_{50}, the P_{O_2} values were corrected to *p*H 7.40 using the Severinghaus nomogram[7].

The effect of DBA on the smooth muscle was tested on isolated guinea pig ileum; contraction occurred with concentrations greater than 1×10^{-8} g ml^{-1}. Finally, its effect on blood pressure was tested using the rat cardiovascular system. Intravenous injection of doses less than 1 mg kg^{-1} resulted in transitory hypertensive action (about 15–18 mmHg), whereas injection of more than 5 mg kg^{-1} produced transitory hypotensive action (about 25 mmHg).

The *in vitro* properties of DBA described here, together with the lack of acute toxicity, suggest that this compound may be a useful thereapeutic agent. Further studies along these lines and on other compounds related to DBA are in progress.

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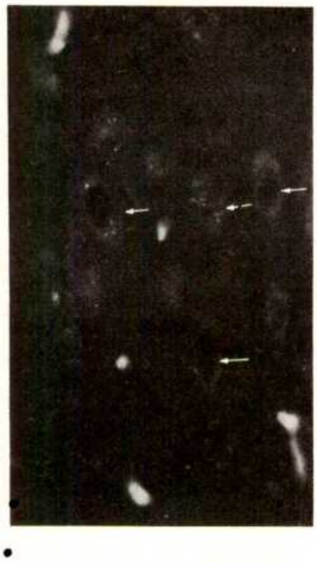
Immunohistochemical localisation of S–100 protein in brain

IT is accepted that the brain-specific protein S–100 is associated predominantly with glial cells[1–5]. More equivocal evidence exists to suggest a possible relationship of S–100 to neurones[3,6,7]. Although well characterised antisera against S–100 have long been available[8], few immunohistochemical studies of the location of S–100 antigen in brain have appeared. Two such studies have reported that S–100 is associated with neuronal elements[9,10] but in both cases the major glial fraction of S–100 does not appear to have been localised by the immunohistochemical techniques used. The recent study by Haglid *et al.*[10] claimed to demonstrate that S–100 antigen is associated with the junctional membranes of brain synapses, and on this basis an ambitious theory of chemical events underlying "learning" has been proposed[11] in which S–100 is assigned a key role. We have now reinvestigated the distribution of S–100 in brain tissue using a specific antiserum against S–100 (a generous gift from Dr L. Levine) which gave a positive response against rat brain extract at 1 : 1,400 dilution in complement fixation assay. The immunological properties of this antiserum have been described in detail by Kessler *et al.*[12].

Brain tissue was obtained from rats fixed by transcardiac perfusion with 4% formaldehyde in 0.1 M phosphate buffer (*p*H 7.4; immunofluorescence procedure) or the same fixative with the addition of 0.5% glutaraldehyde (peroxidase procedure). Cryostat sections (10 μm) were used in immunofluorescence procedure while tissue slices were used for the immunoperoxidase procedure, cut on a Mickle-McIlwain tissue chopper set at 100 μm. In both cases tissue samples were first incubated at room temperature with a 1 : 50 dilution of non-immune serum or one of the antisera, washed in three changes of PBS (0.15 M sodium chloride in 0.1 M phosphate buffer *p*H 7.4) and reincubated as before with either fluorescein-labelled sheep anti-rabbit IgG or peroxidase labelled goat and anti-rabbit IgG. After washing with PBS the peroxidase reaction was developed with 5 mg of diaminobenzidine in 10 ml of PBS containing 1 μl of 30% hydrogen peroxide. The electron microscope samples were postfixed in 1% osmium tetroxide, dehydrated in alcohols and embedded in Araldite. The immunofluorescence procedure shows that non-immune serum does not label brain tissue (Fig. 1) which after this treatment exhibits only weak nonspecific fluorescence with some bright yellow autofluorescent granules in large neuronal perikarya. In contrast anti-S–100 induces labelling throughout the brain in cells which from their morphology and distribution are readily identified as glia. In the cerebral cortex (Fig. 2) astrocytes are selectively revealed, whereas in the cerebellar cortex (Fig. 3) it is the Bergman glial cells and their radial

Fig. 1 Cryostat section of rat cerebral cortex after treatment with non-immune serum by the immunofluorescence procedure described in the text. No immunofluorescent labelling is visible. Autofluorescent granules appear in pyrimidal cell bodies (arrows). Magnification, ×330.



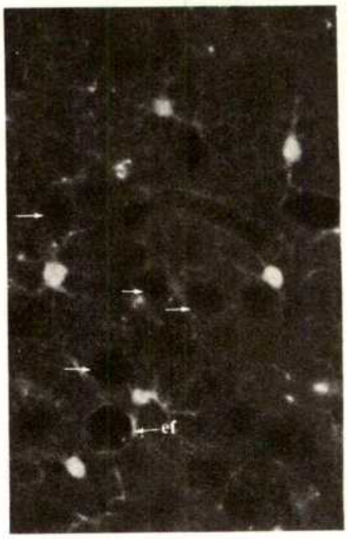
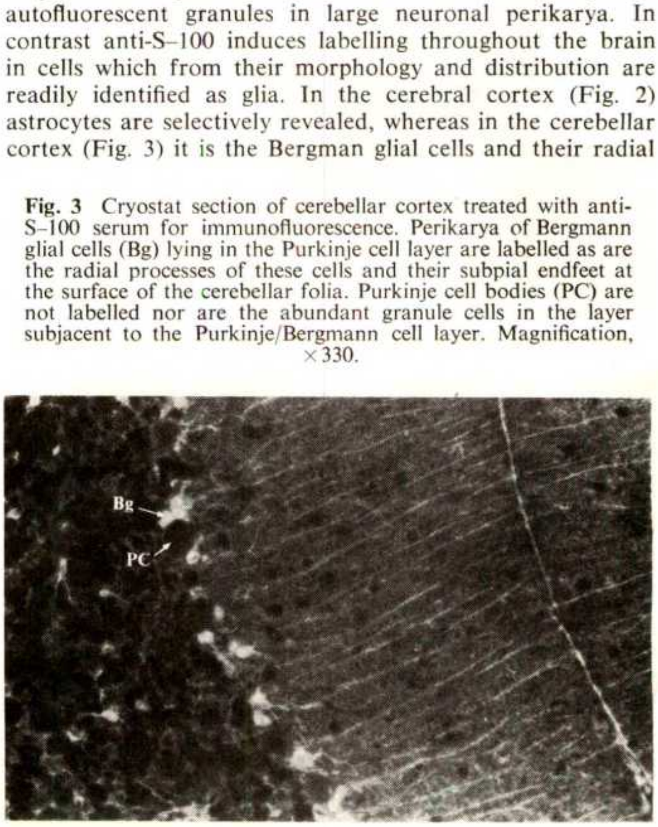


Fig. 2 Cryostat section of rat cerebral cortex treated with anti-S–100 serum for immunofluorescence. Immunofluorescent labelling is present in scattered cells whose morphology and distribution conform to that of glial astrocytes. Astroglial endfeet (ef) on blood vessels are also labelled. Fluorescence is notably absent from neuronal cell bodies (arrows). Magnification, ×330.

Fig. 3 Cryostat section of cerebellar cortex treated with anti-S–100 serum for immunofluorescence. Perikarya of Bergmann glial cells (Bg) lying in the Purkinje cell layer are labelled as are the radial processes of these cells and their subpial endfeet at the surface of the cerebellar folia. Purkinje cell bodies (PC) are not labelled nor are the abundant granule cells in the layer subjacent to the Purkinje/Bergmann cell layer. Magnification, ×330.



processes which are strikingly labelled. In all brain regions glial cells and their processes are also selectively stained by immunoperoxidase histochemistry with anti-S–100 (Fig. 4) by which means two classes of glial cells can be distinguished: those whose nuclei are stained by anti-S–100 and those whose nuclei are not so stained. With either immunohistochemical method no labelling of neuronal cell elements by anti-S–100 could be detected at the light microscopic level.

In the electron microscope, immunoperoxidase histochemistry is again capable of revealing glial cells and their processes, which are filled with the electron-dense peroxidase reaction product (Fig. 5). In neither cerebral nor cerebellar cortices did we find any neuronal cell bodies or processes, either axonal or dendritic, containing anti-S–100 induced reaction product.

We have paid particular attention to the postsynaptic junctional membrane where Haglid *et al.*[10] claim to have localised S–100 antigen by immunoperoxidase histochemistry. The detection of electron-dense reaction product at this site is complicated by the presence of endogenous structures, the postsynaptic densities[13,14], which as their name implies do themselves appear electron-dense after routine fixation for electron microscopy. To distinguish between two types of electron density at the same site is obviously difficult, and to maximise the difference between anti-S–100-induced peroxidase reaction product and endogenous synaptic density we have omitted the customary staining with uranyl or lead salts, both of which enhance the electron density of the postsynaptic density[14]. In these conditions it can be seen that although astrocytic processes in the neuropil, including those which invest the synapse, contain abundant anti-S–100 induced peroxidase reaction product, the synaptic junctional densities show only their endogenous density (Figs 5 and 6). Indeed the intimate relationship between glial processes and synaptic junctions is strikingly demarcated by this method, and astrocytic elements are seen to contain S–100 antigen where they surround both axon terminal and dendritic spine up to their abutment against the synaptic cleft (Fig. 6).

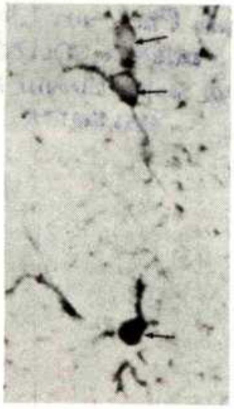


Fig. 4 Tissue slice of cerebral cortex treated with anti-S–100 in the immunoperoxidase procedure described in the text. Three glial cell bodies in this field show the immunoperoxidase stain (arrows) in one of which the nucleus is also stained. Various stained cellular processes are also visible. Magnification, ×400.

Our results thus contradict those of Haglid *et al.*[10] in showing a complete absence of S–100 antigen from synaptic junctions in brain. Since glial S–100 in astrocytic processes immediately adjacent to the synaptic junction was visualised by our staining procedure, the apparent absence of S–100 from the junctions in our preparations cannot be attributed to failure of the staining reaction. On the other hand Haglid *et al.* have not presented evidence that their immunohistochemical preparations are capable of revealing the glial S–100 which constitutes the major portion of this protein in brain tissue. Since the glial S–100 has not been localised, their claimed anti-S–100 staining of the postsynaptic membrane must be subject to question.

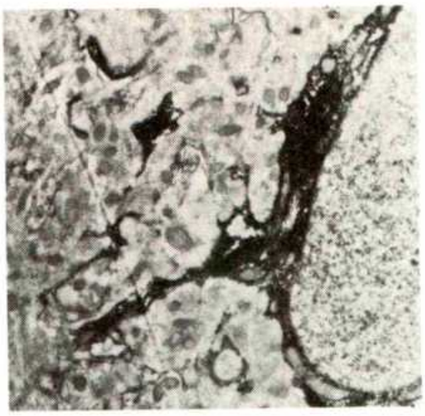


Fig. 5 Electron micrograph of cerebral cortex tissue stained with anti-S–100 by the immunoperoxidase procedure. A glial cell body and its processes are filled with immunoperoxidase reaction product. Magnification, ×5,000.

Absence of S–100 from synaptic junctions is supported by available biochemical evidence. After reduction of disulphide bonds S–100 protein has a mobility in chromatographic procedures indicative of a molecular weight of 7,000 daltons (ref. 15). Electrophoretic analysis of isolated synaptic membranes and isolated synaptic junctional densities shows that protein components in this molecular weight range are absent[16,17].

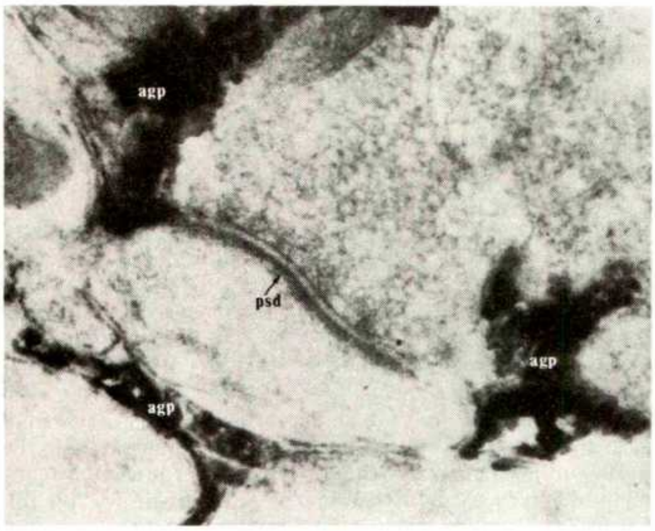


Fig. 6 A synapse in the cerebral cortex neuropil showing the staining obtained at all such sites with anti-S–100 by the immunoperoxidase procedure. Astroglial processes (agp) surrounding the axon terminal and dendrite are stained but the neuronal components of the junction are unstained. The junctional membranes show only their endogenous dense staining structures. No immunoperoxidase reaction product is associated with the postsynaptic density (psd). Magnification, ×62,500.

Our results suggest that S–100 in brain is exclusively associated with glial cells. This implies that S–100 is not directly involved in chemical events occurring within neuronal cytoplasm or the synaptic membranes, although its presence in glial elements contiguous with the junction may be functionally significant. In particular the selective interaction which S–100 undergoes with Ca^{2+} and other cations[18,19] and the importance of Ca^{2+} in synaptic transmission may have some bearing on its presence at this site.

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MATUS and Mughal report a failure to localise S–100 protein in neuronal structures by the aid of immunofluorescence and immunoelectron microscopy. At present, the localisation in neuronal structures of the S–100 protein has been reported in 14 papers[1-7].

In describing their technique, Matus and Mughal state that they have used a "non-immune serum" diluted 1:50 for controls, and found only weak cytoplasmic autofluorescence of neurones with the immunofluorescence procedure. This is hardly a sufficient control since such procedures require careful testing of the experimental conditions as a basis for interpretation. For controls, they should have used antiserum against S–100, from which antibodies against S–100 had been removed by affinity chromatography or by absorption procedures[1-7]. Seeing that immunohistochemical tests for the S–100 protein were used by Matus and Mughal, their negative results are open to doubt.

We would like to question another detail in their technique. Matus and Mughal have fixed the brain tissue with 4% formaldehyde and 0.5% glutaraldehyde (in the peroxidase procedure). This treatment will probably abolish the S–100 antigen–antibody reaction. We have found that 0.5% glutaraldehyde used for fixation of brain tissue for 1 h followed by thorough rinsing was detrimental to this antigen–antibody reaction[7]. Immunoelectrophoresis demonstrated furthermore that 0.5% glutaraldehyde added to a solution of S–100 protein completely inhibited S–100 antigen–antibody precipitation. Formaldehyde, 4%, decreased the reaction by 60% (ref. 7).

In their technical description Matus and Mughal do not report which measures and tests they have used to secure the specificity of the antiserum to S–100. We have stressed[3-7] the need to prepare a pure antigen (S–100) against which the antiserum can be tested. The purity of the S–100 was checked in sucrose density gradients and by electrophoretic methods. The antigen thus obtained was used to test the specificity of the antiserum to S–100. This specificity was tested in electrophoretic systems and by complement fixation[1-7].

Matus and Mughal point out that detection of differences in electron density at the site of the postsynaptic membrane is complicated. We fully agree and consider that the only interpretable result could be judged, as the difference in electron density between sections treated with an antiserum containing antibodies against S–100 protein, and the same antiserum absorbed repeatedly with the antigen. This would ensure that the proper antibody has been studied in sufficient concentration.

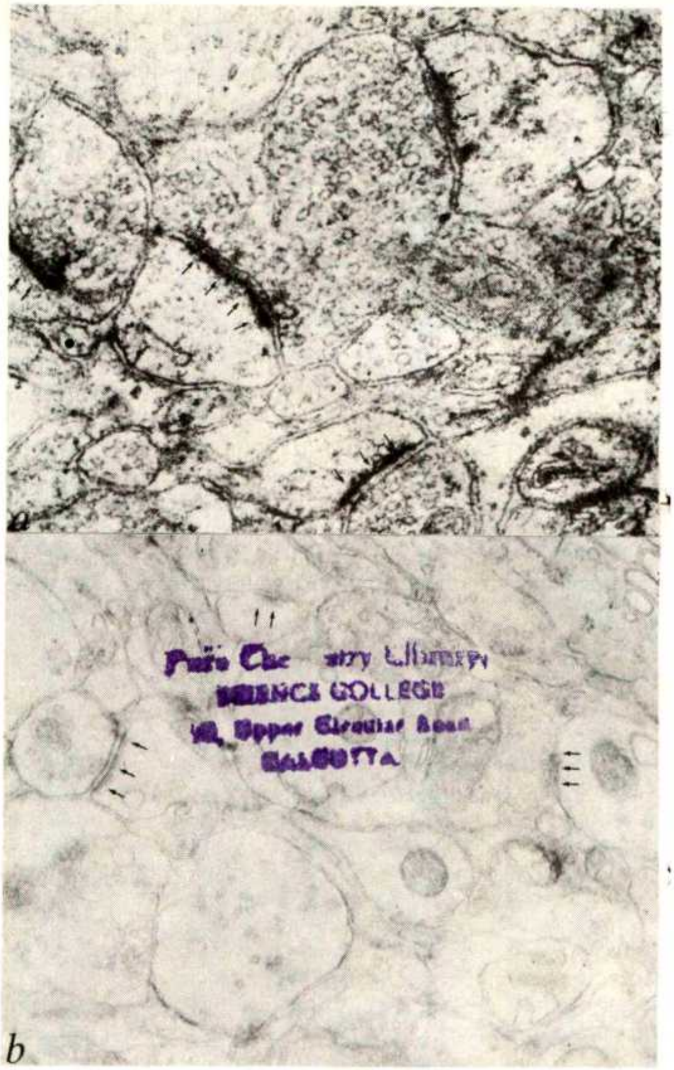

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Fig. 1 *a*, Synapse incubated in anti-S–100 antiserum conjugated with peroxidase. Heavy anti-S–100 peroxidase activity is seen in the postsynaptic membrane (arrow) (×36,040). *b*, Synapse incubated in anti-S–100 antiserum conjugated with peroxidase and repeatedly absorbed with pure S–100. No precipitates are seen in the postsynaptic membrane (arrow) (×36,040).

In ref. 1 we studied exclusively neuronal S–100. The reason why we did not demonstrate much glial S–100 in subsequent work[7] is that most soluble S–100 is lost to the medium from the cells during the mild fixation employed by us. Thus, the medium seems brownish after DAB incubation. We feel it necessary to stress that in our preparation only the small membrane-bound (less than 10%) portion of the total S–100 in the tissue is demonstrated.

Figure 1*a* shows a synapse from adult rat frontal cortex incubated in anti-S–100 antiserum conjugated with peroxidase. A heavy anti-S–100 preoxidase activity is seen in the postsynaptic membrane. Figure 1*b* shows a similar synapse incubated in anti-S–100 antiserum conjugated with peroxidase and repeatedly absorbed with pure S–100. No precipitates are seen in the postsynaptic membrane.

Our data do not, however, fully contradict Matus and

Mughal's results since some synapses do seem to lack S–100 activity[7].

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Tyramine activates the EEG in epileptic patients

IT has been suggested that abnormalities of monoamine metabolism may be important in migraine[1], and Sandler *et al*[2] have demonstrated a reduction in monoamine oxidase activity in these patients. This observation might explain Hanington's finding[1] that an oral tyramine load could induce headache in selected individuals. These subjects had noticed that certain foods known to contain tyramine, such as cheese, regularly induced their migraine headaches. Although unable to confirm that single doses of oral tyramine regularly precipitated headache[3] we have shown that this amine accentuates pre-existing electroencephalogram (EEG) abnormalities in subjects with dietary-induced migraine alone, and in a group of patients who had both migraine and epilepsy, but not in a group with non-dietary migraine. These observations suggested that the effect of tyramine on the EEG should be studied in patients with epilepsy alone. Furthermore, a preliminary investigation had indicated that tyramine caused an increased number of spikes in the EEG recordings of monkeys with alumina-induced epileptic foci[4].

We studied the effect of tyramine on the EEGs of a group of 15 patients with epilepsy using a double-blind placebo-controlled design. None of these subjects had migraine. Six women and nine men (mean age 36; range 16–63 yr) agreed to participate after the investigation had been explained to them. They suffered from generalised epilepsy of major or minor type or from temporal lobe epilepsy (Table 1). In most patients seizures were not well controlled. During the period of the investigation their usual anti-convulsant medication was continued. The trial required each patient to take a capsule on two separate occasions at least 1 week apart. One capsule contained tyramine hydrochloride (125 mg), and the other contained lactose placebo. EEGs were recorded approximately 5 h after ingestion of each capsule. All the recordings were made in a standard manner, at the same time of day, usually by the same technician, and using the same apparatus. During the 24 h after taking the test substances none of the 15 subjects complained of headache and none experienced seizures.

The 15 pairs of EEGs were masked, coded and randomised. Each pair was independently assessed by two raters using a previously determined procedure[4,5]. In particular, disturbances of background activity and the occurrence of paroxysmal features, including spike activity, sharp waves, and spike and wave, were noted. All the EEGs were abnormal. The two raters agreed as to which was the more abnormal of a pair in 14 of the 15 instances. When the EEGs were unmasked it was found that in 11 of the 14 agreed assessments the recording made after the ingestion of tyramine was the more abnormal of the pair ($P<0.01$). Accentuation of EEG abnormality after tyramine occurred both in patients with generalised epilepsy and in those with focal epilepsy (Table 1).

Table 1 Activating effect of tyramine on EEG of epileptic patients

	Activated	Not activated
Generalised epilepsy	5	0
Temporal lobe epilepsy	6	3
Total	11	3

$n = 14$

There are two separate problems in epilepsy to which these observations might be related. First, in epileptic patients there is a continuing subclinical disorder that is shown by the presence of abnormalities in inter-ictal EEG recordings and, second, there must be a triggering mechanism which induces clinical seizures at a particular moment. The underlying functional disturbances accounting for both these factors is uncertain although abnormalities of the cellular sodium pump[6] and γ-aminobutyric acid metabolism[7] have been suggested in experimental work. The present study showed that activation of pre-existing focal or generalised 'epileptic' EEG abnormalities was induced by oral tyramine. Although the type and quantities of these abnormalities are known to bear a direct relationship to seizure incidence[8,9] none of our patients experienced seizures in the 24 h after ingestion of tyramine. We have not yet studied the effects of different doses of tyramine in these or other epileptic subjects.

Tyramine is a substrate for monoamine oxidase metabolism and it is possible that our findings could be related to a disturbance of that enzyme. This suggestion is supported by evidence that the seizure threshold in epileptic patients may be modified by changes in cerebral monoamines and that anti-convulsant drugs alter cerebrospinal fluid monoamine metabolite concentrations[10]. It is further supported by the fact that monoamine oxidase inhibitor drugs may precipitate seizures in some individuals[11] and by reported abnormalities in this enzyme in patients with another paroxysmal disorder of the brain, migraine[2]. This is also a disorder in which EEG abnormality is present[4]. Disturbance of brain monoamine oxidase metabolism probably also has an important role in endogenous depression[12] and schizophrenia[13], other disorders in which EEG abnormalities may occur.

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Disruption of brain stimulation-induced feeding by dopamine receptor blockade

It is well established that electrical stimulation of the lateral hypothalamus can be used to elicit a variety of biologically significant behaviour[1,2]. For many years, this behaviour was usually attributed to the artificial activation of neural systems which control consumatory responses, but this interpretation has been called into question[3]. The criticism arises in part from behavioural experiments in which stimulation-induced feeding and drinking were shown to differ from their natural counterparts in a number of important respects[4,5], and also from neuropharmacological studies (ref. 6 and A G P. and H C. Fibiger, unpublished) showing that food deprivation and brain stimulation-induced feeding or drinking were differentially affected by destruction of catecholamine (CA) systems in the brain. These latter results clearly implicated brain CAs in a stimulation-induced behaviour and as such emphasise the parallels between electrically-induced behaviour and stereotypies which accompany high doses of amphetamine[7].

Amphetamine increases the release and prevents reuptake of both noradrenaline (NA) and dopamine (DA) and could therefore induce stereotypy by its action on any of the brain CA systems[8–10]. DA seems, however, to play a critical role in amphetamine stereotypy as this drug-induced behaviour is abolished by lesions of the dopaminergic nigroneostriatal bundle[11,12]. Furthermore, a similar class of stereotypical behaviour is elicited by the DA agonist apomorphine[13]. Therefore, if brain stimulation and drug-induced behaviour are subserved by a common neurochemical substrate, the former behaviour should be disrupted by treatment with a DA antagonist. As a test of this hypothesis, we observed the effects of the neuroleptic drug haloperidol on feeding elicited in satiated rats by electrical stimulation of the lateral hypothalamus. In small doses, haloperidol has been reported to block central DA receptors[14].

Twenty-five male Wistar rats had bipolar electrodes implanted into the lateral hypothalamus under stereotaxic control. Implantation procedure and behavioural testing were carried out as described previously[6]. Briefly, the behavioural testing consisted of first selecting a current intensity which induced forward locomotion and searching, and observation of each animal for five daily test sessions, consisting of twenty 30-s periods of brain stimulation, separated by 60-s interstimulation intervals. The six animals which showed brain stimulation-induced feeding behaviour during seven or more stimulation periods on a given day, were retained for further testing.

During the next phase of the experiment, these six animals were tested for 5 d per week. Three days of testing preceded the drug treatment, and one day of testing followed it. On day 3 of each week, the animals were injected with saline (0.9%), on day 4 they were injected intraperitoneally with either 0.05, 0.10 or 0.15 mg kg^{-1} haloperidol (Haldol, McNeil Laboratories) 45 min before beginning the test series. Measurements of the total time engaged in stimulation-induced behaviour, and the amount of wet mash ingested were taken, together with the number of trials on which the behaviour was displayed. During the next week, the effect of haloperidol (0.15 mg kg^{-1}) on deprivation-induced food intake, was observed. The amount of food consumed in 1 h following 23 h food deprivation was measured for 4 d and on the day 5, the food intake after pretreatment with haloperidol was monitored. At the end of week 4 of testing, the animals were asphyxiated in CO_2, their brains removed, sectioned on ice, and electrode placements confirmed microscopically. The six electrodes were all located in the lateral hypothalamus, with five situated dorsolateral to the fornix, on the edge of the cerebral peduncle.

The data from each of the three measures are shown in Table 1. Treatment with the DA receptor blocker haloperidol produced a disruption of stimulation-induced feeding, which followed a clear dose–response relationship. None of the three measures of stimulation-induced feeding was affected by the lowest dose of haloperidol (0.05 mg kg^{-1}) and only a slight decrease was observed after 0.10 mg kg^{-1}. A significant decrement accompanied the highest dose (0.15 mg kg^{-1}), however, as reflected in a 74% reduction in incidence ($P<0.01$), 76% reduction in time ($P<0.01$) and 70% decrease in amount of food consumed ($P<0.01$). In contrast, this same dose of haloperidol had no significant effect on the feeding behaviour of deprived animals, confirming a recent report[15]. Food intake during the no-drug condition was 35 g wet mash, whereas the

Table 1 Effect of haloperidol on stimulation-induced feeding as measured by incidence, time and amount of food consumed

Measures	Treatment Saline	Haloperidol (0.05 mg kg^{-1})	Saline	Haloperidol (0.10 mg kg^{-1})	Saline	Haloperidol (0.15 mg kg^{-1})
Incidence of stimulation-induced feeding (maximum 20)	13±4.4	12.8±6.9	13.6±4.1	10.2±6.8	11.3±3.8	3.0±1.9*
Total time engaged in stimulation-induced feeding (maximum 600 s)	81.7±36.2	86.2±65.2	90±40.8	58.6±47.8	57.5±20.8	13.8±10.7*
Amount of food (g) consumed during 20 stimulation periods	25.5±9.2	26.5±17.5	27.2±11.6	20.3±11.9	21.5±4.8	6.5±4.3*

Data expressed as mean ± s d

$P < 0.01$ when compared with saline by analyses of variance and *post hoc* Student's *t* test.

same animals consumed 30 g in 1 h following 0.15 mg ml^{-1} haloperidol ($P>0.05$).

Previous experiments have implicated brain CAs in stimulation-induced behaviour and the present results suggest an important function for DA. Dopaminergic mechanisms are thought to be involved in the homeostatic regulation of food and water intake[16–18], although the precise nature of this involvement is unknown. Theoretically, feeding and drinking are controlled by a number of different subsystems and in this context, it is of interest to note that whereas haloperidol has no effect on food deprivation-induced feeding, eating in response to insulin is attenuated by this drug[6]. It is, therefore, conceivable that brain stimulation-induced feeding may be subserved by activating one or more specific subsystems normally involved in the regulation of food intake. If brain stimulation-induced feeding is in some way dependent on neural systems controlling *ad libitum* food intake, however, the relationship seems to be a limited one in view of the differential effects of both haloperidol and 6-hydroxydopamine (ref. 6 and A.G.P. and H. C. Fibiger, unpublished) on these different types of feeding behaviour.

As an alternative explanation, it is compelling to consider brain stimulation-induced behaviour as representative of a general class of stereotypical behaviours which include drug-induced stereotypies[7,13] and consumatory responses evoked by mildly arousing stimuli such as tail-pinch in rats[19]. Compulsive oral movements including licking and gnawing, are characteristic of each class of induced behaviour and these responses are attenuated by disruption of dopaminergic activity regardless of the type of stimulation used to evoke them. Although the present data do not permit specification of a particular dopaminergic substrate for brain stimulation-induced behaviour, both tail-pinch and amphetamine-induced stereotypies seem critically dependent on the nigroneostriatal bundle[12,13,19]. This system may also be important for electrically-induced behaviour, as preliminary results in our laboratory indicate that such behaviour is disrupted by neurotoxic lesions of the nigroneostriatal bundle. It has been suggested that the nigroneostriatal bundle serves an important gating function in the control of neostriatal motor output[20], and therefore disruption of this system could result in the release of prepotent motor responses normally inhibited by the neostriatal bundle. Valenstein[21] has proposed that behaviour induced by brain stimulation, tail-pinch and amphetamines could represent coping responses initiated by a state of high arousal and hyper-responsiveness to stimuli, resulting from facilitation of DA pathways. Although these explanations undoubtedly represent an oversimplified statement of the role of dopamine in stereotype behaviour, this perspective could provide important insights into stereotype behaviour in general, and brain stimulation-induced behaviour in particular.

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Organisation of receptors for neurotransmitters on *Aplysia* neurones

THE application of acetylcholine (ACh) to neurones of *Aplysia* has shown the existence of three different responses, mediated by conductance increases to Na$^+$, Cl$^-$ and K$^+$ respectively[1]. These neurones also have receptors for various other putative neurotransmitters, including serotonin[2], dopamine[3], octopamine[4,5], phenylethanolamine[5], γ-aminobutyric acid[6,7], glutamic acid[6,7], aspartic acid[7] and histamine[5,8]. With one possible exception there are at least three types of response to each of these substances resulting from conductance increases to Na$^+$, Cl$^-$ and K$^+$, respectively (refs 5–7 and our unpublished observations). These facts suggest that specific receptors (neurotransmitter-binding sites) and ionophores for Na$^+$, Cl$^-$ and K$^+$ might be building blocks which can be assembled in any combination. If so there should be common properties of the receptors for one transmitter mediating the three different ionic responses, as well as similarities in the responses to different transmitters when the conductance change is to the same ion. In this report we compare the three dopamine and ACh responses as a test of this hypothesis.

Neurones were penetrated with double-barrelled glass microelectrodes as previously described[4,8,9]. One barrel was used to record membrane potential and the other to pass d.c. or current pulses for measurement of membrane resistance. Drugs were iontophoresed using a five-barrelled extracellular micropipette. The iontophoretic control unit was designed to pass the same total charge through each of the five barrels, and would automatically vary pulse duration to achieve constant total charge (J. A. Willis, in preparation). For substances with similar transport numbers this method allows a comparison of the sensitivity of a receptor to equal amounts of different substances applied to a localised site.

Figure 1 shows examples of the three different conductance increase responses to dopamine. Each of these three neurones was studied with iontophoretic electrodes containing noradrenaline, octopamine and phenylethanolamine in addition to dopamine. Figure 1*a* shows a neurone responding to dopamine with a large depolarisation which causes a brisk discharge. This neurone also responds to noradrenaline but not as well as to dopamine. In the absence of Na$^+$ (replacement with the impermeant Tris$^+$) there is no response, indicating that the depolarisation is a result of a conductance increase to Na$^+$. In Fig. 1*b* another neurone is hyperpolarised best by dopamine and less well by noradenaline. This response is due to a Cl$^-$ conductance increase, since removal of extracellular Cl$^-$ (replacement by impermeant acetate) causes a reversal of the polarity of the response due to the reversal of the Cl$^-$ concentration gradient. In another neurone (Fig. 1*c*) a similar relative sensitivity to dopamine and noradrenaline results for a response unaffected by removal of extracellular Cl$^-$ and identified by Ascher[3] as being due to a K$^+$ conductance increase In all three neurones there was no response to octopamine or phenylethanolamine although other neurones have receptors for these substances[4,5]. Adrenaline, applied to the bath at a concentration of 10^{-4} M, caused a con-

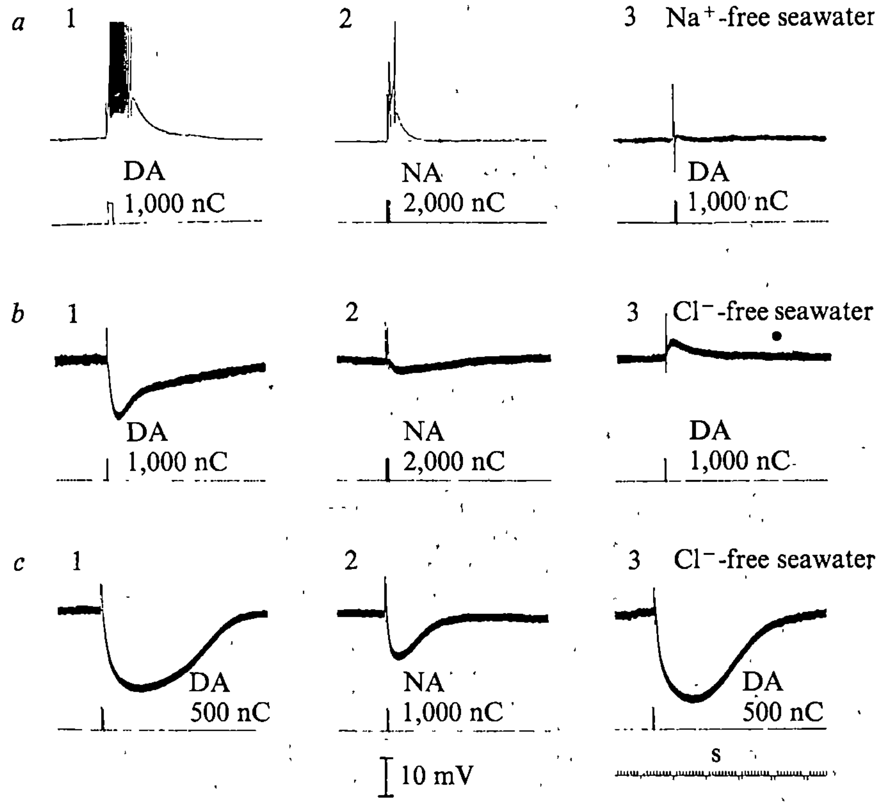

Fig. 1 Effects of iontophoretic application of dopamine and noradrenaline on to three different unidentified neurones from the abdominal (*a* and *b*) and cerebral (*c*) ganglia. The upper recording in each trace is from the intracellular micropipette, whereas the lower trace shows the duration of the iontophoretic pulse. The total charge passed by iontophoresis is indicated near the pulse. In *a*3 the ganglion was perfused with seawater where Na^+ was replaced by $Tris^+$, whereas in *b*3 and *c*3, all Cl^- was replaced by acetate. The time calibration applies as shown only to *a* and *b*. In *c* the time base is slower by a factor of 2. DA, dopamine; NA, noradrenaline.

ductance change similar to but smaller than that caused by dopamine and noradrenaline. In every case, membrane resistance fell during the response. Figure 2 shows that when the dopamine receptor is desensitised there is no longer a response to noradrenaline. We conclude that both substances are acting on a common receptor, and that the structure–activity relation of that receptor is identical for all three ionic responses.

Table 1 gives the average time-to-peak of the Na^+, Cl^- and K^+ responses to dopamine and ACh. Neurones were unselected except for the requirement of clear identification of the ionic conductance involved by changing the seawater composition as illustrated in Fig. 1 and/or by obtaining an inversion potential at close to levels previously reported[1] (−60 mV for E_{Cl^-}, −80 mV for E_{K^+}). For both dopamine and ACh the Na^+ and Cl^- responses are very much more rapid than the K^+ responses. The values for the same ionic conductance to the different transmitters are very similar.

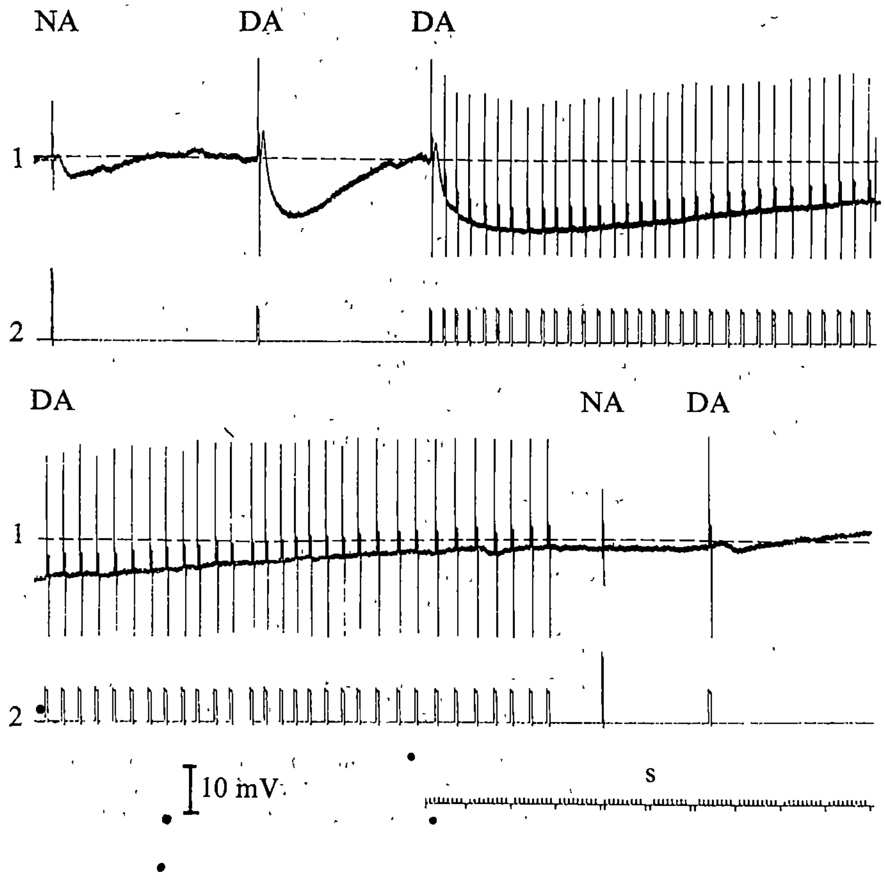

Fig. 2 Effects of dopamine receptor desensitisation on the response of a pleural neurone to noradrenaline. Intracellular recordings are shown in trace 1, whereas trace 2 indicates the iontophoretic pulses. The upper and lower traces are continuous. In trace 2 the large pulse indicates noradrenaline application while the smaller one indicates dopamine. The total charge was 1,000 nC for both dopamine and noradrenaline. Multiple pulses of dopamine resulted in a hyperpolarisation which decayed towards control potential with time. In these conditions membrane resistance returned to control values. When the receptor was desensitised to dopamine there was no longer a response to noradrenaline, indicating that both act on the same receptor.

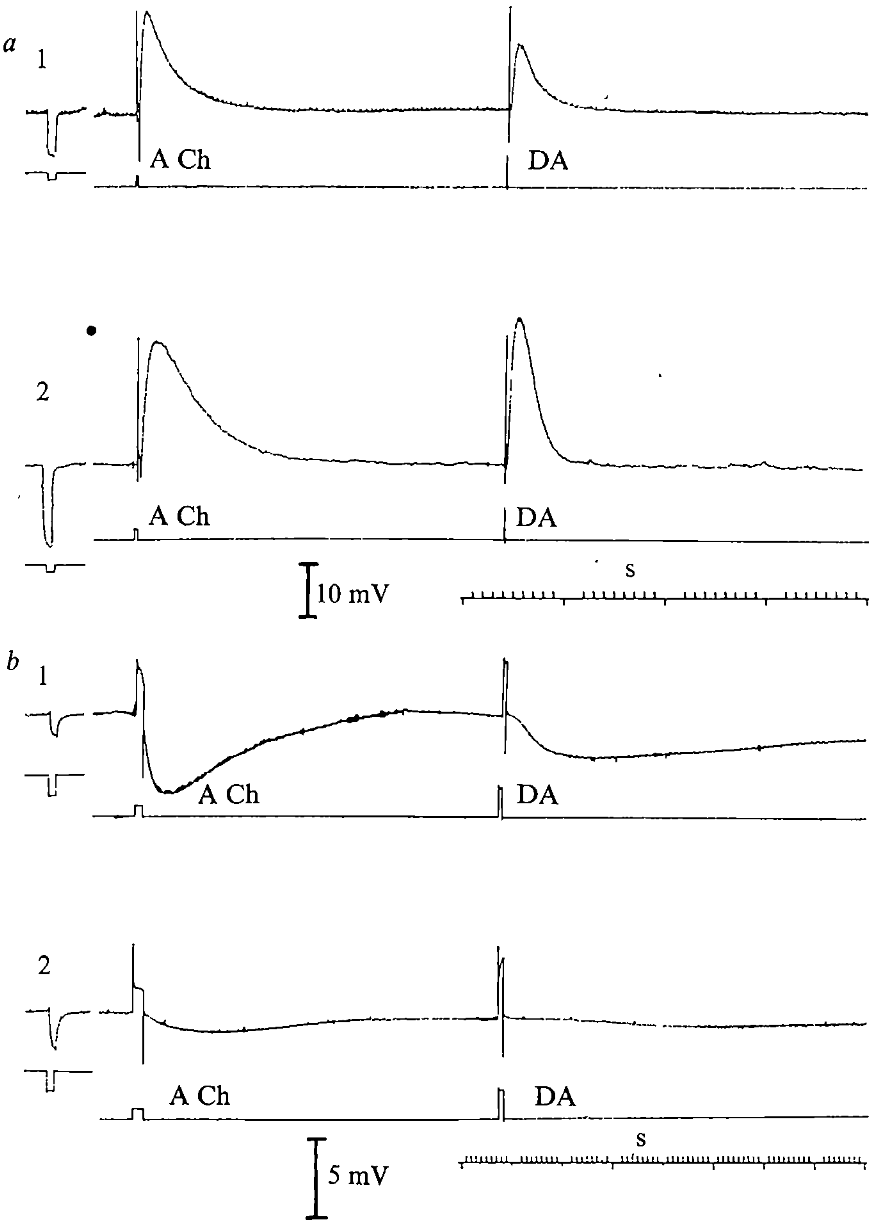

Fig. 3 Effects of temperature on Na$^+$ and K$^+$ responses to ACh and dopamine. In each recording the upper trace is the intracellular potential and the lower is the indication of the iontophoretic pulse. The small inserts at the left of each row show a measure of membrane resistance (upper) to a current pulse of 1.2×10^{-8} A in (*a*) and 4.0×10^{-8} A in (*b*). Records *a*1 and *b*1 were made at 24 °C, and records *a*2 and *b*2 at 8 °C Temperature changes and methods of recording were as previously described[11]. The neurone in (*a*) was from the anterior pleural group described by Kehoe[1], whereas (*b*) was from cell R_{14} in the terminology of Frazier *et al.*[12]. For both neurones penetrations were made with two independent intracellular electrodes (one for recording, the other to pass current). Iontophoretic current was 100 nC in (*a*) and 1,000 nC in (*b*) for both dopamine and ACh. For R_{14} penetration and iontophoresis were made through the capsule.

In all cases the dopamine response is slightly slower than that to ACh, but this, in part at least, reflects the fact that all dopamine receptors are located in the neuropil (in agreement with Ascher[3]), whereas ACh receptors are both on soma and neuropil.

The ionic selectivity of the K$^+$ ionophore has been studied for both ACh and dopamine receptors. Kehoe[1] has shown that ACh K$^+$ ionophore is impermeable to Cs$^+$ but one half as permeable to Rb$^+$ as K$^+$. Ascher[3] has found a similar pattern for the dopamine K$^+$ ionophore. In addition, Kehoe[1] has demonstrated that intracellular TEA injections block the ACh K$^+$ responses (presumably by occluding the K$^+$ channel[10] rather than by blocking the ACh receptor complex, as it does when applied extracellularly). Ascher[3] demonstrates a similar sensitivity of the dopamine K$^+$ ionophore to intracellular but not extracellular TEA

Figure 3 illustrates the temperature dependences of Na$^+$ and K$^+$ responses. The neurone in Fig 3*a* gave a Na$^+$ conductance to both ACh and dopamine. On cooling from 23 °C (1) to 8 °C (2) the membrane resistance increased considerably and the responses increased proportionately. In Fig. 3*b*, K$^+$ responses to both ACh and dopamine were reduced considerably by cooling in spite of an increased resistance. These results are in agreement with others[1,3]. Although we have found exceptions to the generalisation that all Na$^+$ responses are relatively temperature insensitive and all K$^+$ responses are blocked by cooling, the temperature dependence of the same ionic responses elicited by two different transmitters on the same cell has always been the same.

Shain *et al.*[9] suggested that *Aplysia* contains a single ACh receptor on the basis of observations that α-bungarotoxin blocks all three ACh responses and binds to a membrane homogenate with a single association and dissociation constant Our observation that the various phenylethylamines have similar relative effectiveness in activating the three dopamine responses is consistent with the view that the dopamine binding site is identical irrespective of the conductance change elicited. The similarities in time course,

Table 1 Times-to-peak of Na$^+$, Cl$^-$ and K$^+$ responses to ACh and dopamine

	Na$^+$	Cl$^-$	K$^+$
ACh	1.8±0.2 (36)	2.4±0.2 (43)	12.2±1.7 (6)
Dopamine	2.3±0.7 (14)	2.5±0.5 (7)	15.1±1.5 (21)

Results are expressed in s ± s e. The values in parentheses indicate the number of neurones of each group studied.

temperature sensitivity and ionic selectivity of responses resulting from similar ionic conductance but activation of receptors to different transmitters, suggest that these properties are characteristic of common ionophores.

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Lithium accumulation by snail neurones measured by a new Li$^+$-sensitive microelectrode

THE importance of lithium salts in the treatment of mania[1] has led to considerable research into their physiological and biochemical effects, especially on the nervous system. But, largely because of analytical difficulties, little is known about Li$^+$ accumulation or transport by nerve cells: the available evidence suggests only that there is some Li$^+$ uptake by neurones[2], but little or no active transport of Li$^+$ by the sodium pump[3]. The ideal method for measuring intracellular ions is the ion-sensitive microelectrode, but no electrode suitable for Li$^+$ was available until the recent development of a synthetic Li$^+$-selective ligand[4]; thus we are now able to make Li$^+$-sensitive microelectrodes. Here we describe their construction and use in the direct measurement of intracellular Li$^+$ in snail neurones. We have found that the neurones can maintain lower levels of Li$^+$ inside than out, showing that Li$^+$ must be actively transported out across the cell membrane.

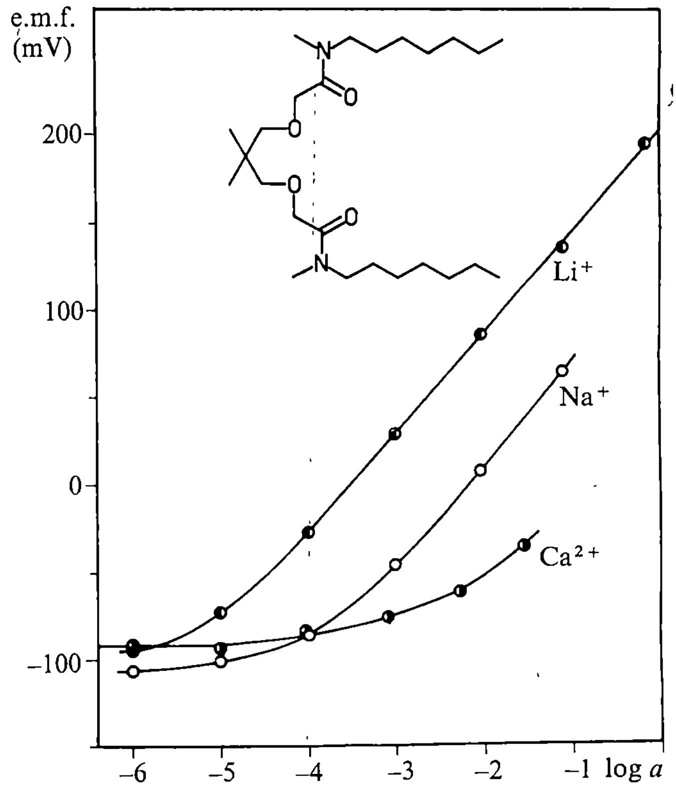
In early experiments, the Li$^+$-selective ligand was used to make macroelectrodes by incorporation in a polyvinylchloride (PVC) membrane using Tris-(2-ethylhexyl)-phosphate (TEHP) as plasticiser. The response characteristics of such a membrane are shown in Fig. 1 (refs 4 and 5). According to Fig. 1, Na$^+$ is discriminated relative to Li$^+$ by a factor $\sim$ 20. So that the same Li$^+$ carrier could be used in microelectrodes with reasonably low ($\sim 10^{10}\,\Omega$) resistances, sodium tetraphenylborate (NaTPB) was added to the membrane phase. The Li$^+$-ligand solution had the following composition (by weight) 9.7% Li$^+$ carrier, 85.5% TEHP and 4.8% NaTPB.

The Li$^+$-sensitive microelectrodes were made from borosilicate glass micropipettes by a modification of one of the methods of Walker[6] (P. Ascher and T. O. Neild, personal communication). The micropipettes were siliconised by dipping for $\sim$ 10 s in a 2.5% v/v solution of tri-*n*-butyl chlorosilane in chloronaphthalene and then heated for at least 20 min at $\sim$ 100 °C. The last few millimetres of the micropipette were filled with the Li$^+$–ligand solution, air bubbles being removed with a cat's whisker (from a real cat) and the rest of the electrode being filled with 2 M LiCl. A chloride-coated silver wire, dipped in the LiCl, completed the microelectrode.

The physiological experiments were done on the largest neurone at the rear of the right pallial ganglion of the snail *Helix aspersa*[7]. The exposed suboesophageal ganglia were superfused with continuously flowing snail Ringer (KCl, 4 mM; NaCl, 80 mM; $CaCl_2$, 7 mM; $MgCl_2$, 5 mM; $NaHCO_3$, 22 mM; Na_2HPO_4/NaH_2PO_4 *p*H 7.5, 0.1 mM; equilibrated with 2.5% CO_2 and 97.5% O_2). The neurone was penetrated with up to four separate microelectrodes: one to measure the membrane potential, one sensitive to Li$^+$, and two for interbarrel iontophoretic injections of LiCl. After an experiment, the Li$^+$-sensitive electrode was calibrated in the experimental bath by the perfusion of solutions with approximately the intracellular levels of Na$^+$ and K$^+$, but different levels of Li. This method avoided the need for complex calculations using selectivity coefficients determined for each electrode. (It should be mentioned that ion-sensitive electrodes measure activity rather than concentration. But since the present experiments were done with an essentially constant ionic strength, the more convenient ion concentrations will be used.)

To check that this calibration method works reasonably well, a Li$^+$-sensitive microelectrode was calibrated intracellularly as well as extracellularly. A series of LiCl injections were made while the internal Li$^+$ concentration ($[Li^+]_i$) was measured with a Li$^+$-sensitive electrode, as shown in Fig. 2. At the beginning of the experiment there was no Li$^+$ inside the cell. When the fourth microelectrode (filled with LiCl) was inserted into the cell there was at first some leakage, but this was stopped by a 5-nA backing current. Five separate 35-nA injections of LiCl were then made, all except the fourth with current duration of 30 s. The neurone diameter was about 200 µm, so that, assuming a spherical cell and a transport number of 1.0, the 30-s injections should each raise $[Li^+]_i$ by 2.6 mM. The observed increases in $[Li^+]_i$ were measured using the extracellular calibration curve. The ratios of the increases in $[Li^+]_i$ calculated from the charge to those measured were 0.96, 1.15, 1.19, 1.21 and 1.15. This suggests that while the cell volume was presumably less than estimated, the

Fig. 1 E.m.f. response of a lithium-selective PVC membrane electrode to aqueous solutions of the chlorides of Li$^+$, Na$^+$ and Ca^{2+}. The e.m.f. values were corrected for changes in the liquid junction potential of the reference electrode (see ref. 4). The structure of the electrically neutral Li$^+$ selective carrier is shown at the top.



Li$^+$-sensitive microelectrode gave very similar responses inside and outside the cell. We found that the Li$^+$-sensitive microelectrodes were usable for at least 2 d after filling, and were very stable in the calibration solutions or intracellularly. But in the snail Ringer the potential of the electrode tended to vary, in particular the potential after a cell penetration tended to differ from that before.

A simple experiment to measure Li$^+$ uptake and loss is shown in Fig. 3. The neurone was initially in normal Ringer. When this was changed to one with half the Na$^+$ replaced by Li$^+$, the Li$^+$ record immediately began to rise, indicating a relatively slow Li$^+$ influx. After about 17 min in Li$^+$ Ringer, the cell stopped firing. When the cell was again exposed to normal Ringer, Li$^+$ ions began to leave the cell. In similar but longer experiments essentially all the accumulated Li$^+$ left the cell within 1 h. The $[Li^+]_i$ at which the spontaneous action potentials ceased was always ~ 5 mM.

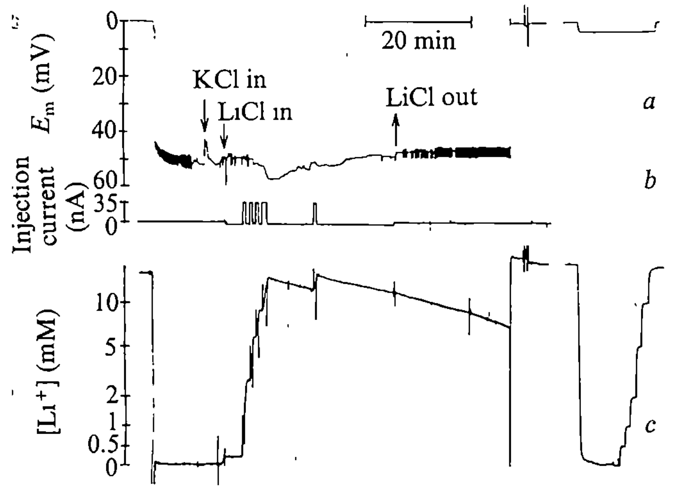


Fig. 2 Recordings comparing the Li$^+$-sensitive microelectrode response to increasing $[Li^+]_i$ inside a neurone with that to calibration solutions. *a*, Potential between the membrane potential (E_m) microelectrode and a reference electrode in the bath; *b*, iontophoretic injection current; *c*, potential between the E_m and the Li$^+$-sensitive microelectrodes The Li$^+$-sensitive microelectrode was inserted first, then the membrane potential microelectrode, and finally two current-passing microelectrodes: one filled with KCl and the other with LiCl. The long time constant of the pen recorder has reduced the size of the action potentials to only a few millivolts. At the end of the experiment the electrodes were withdrawn from the cell and the Li$^+$ microelectrode calibrated by superfusing the preparation with six different calibration solutions These all had the same levels of NaCl (4 mM), KCl (90 mM) and Tris maleate *p*H 7.5 (10 mM), but different levels of LiCl (0, 0.5, 1, 2, 5 and 10 mM).

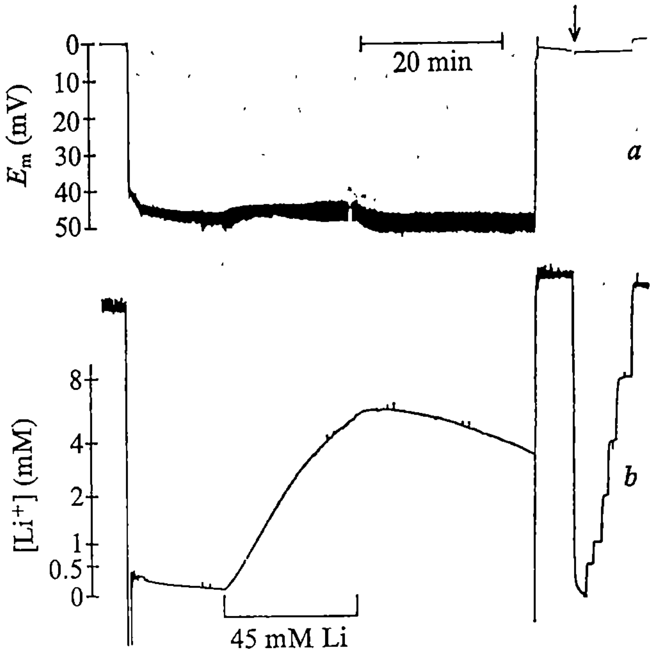
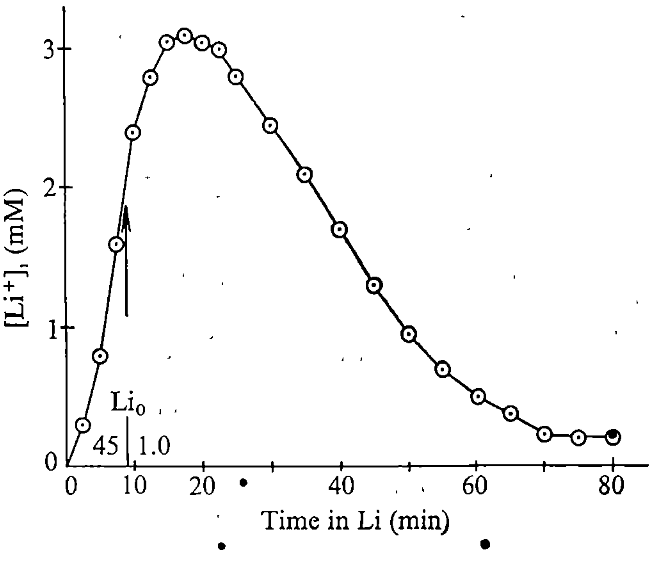


Fig. 3 Li$^+$ uptake by a neurone exposed to 45 mM external Li$^+$. *a*, Membrane potential; *b*, potential of the Li$^+$-sensitive microelectrode. The cell was penetrated by two microelectrodes only. Where indicated, the normal Ringer was changed to one in which half the Na$^+$ was replaced by Li$^+$. At the end of the experiment both electrodes were withdrawn from the cell and the Li$^+$ electrode was calibrated. The six calibration solutions all had the same concentration of NaCl (2 mM), KCl (90 mM) and Tris maleate (10 mM), but different concentrations of LiCl (0, 0.5, 1, 2, 4 and 8 mM). At the point indicated by the arrow the E_m microelectrode blocked and was then broken to a low resistance.

Perhaps the most interesting question that can be easily answered with a Li$^+$-sensitive microelectrode is how much Li$^+$ cells accumulate when exposed to low, 'therapeutic' levels of external Li$^+$. With a purely passive distribution of Li$^+$ across the cell membrane, 1 mM Li$^+$ outside would lead to a $[Li^+]_i$ of 5–10 mM. A number of experiments were therefore done in which cells were loaded with Li$^+$ by a brief exposure to high external levels, and then allowed to equilibrate in Ringer with only 1 or 2 mM LiCl. The graph of $[Li^+]_i$ against time from such an experiment is shown in Fig. 4. After a short exposure to 45 mM Li$^+$ Ringer, the cell was maintained in 1 mM Li$^+$ Ringer until the end of the experiment. Since the membrane potential was ~ 45 mV, a passive distribution of Li$^+$ across the cell membrane would lead to intracellular accumulation of Li$^+$ to a level of more than 5 mM: yet the result shows that $[Li^+]_i$ fell to below 0.3 mM. Thus Li$^+$ was being extruded against both an electrical and a concentration gradient; in some way Li$^+$ was being actively transported out of the cell. This was also seen when cells were exposed to 2 mM or 10 mM external Li$^+$: with 2 mM Li$^+$ outside the steady state $[Li^+]_i$ was < 1 mM, and with 10 mM Li$^+$ outside $[Li^+]_i$ was ~ 5 mM. These results show clearly for the first time that Li$^+$ can be actively transported out of nerve cells. Similarly, frog muscle has recently been found[8] by chemical analysis to accumulate very little Li$^+$ when exposed to low external levels. The mechanism by which Li$^+$ is actively extruded is unknown: perhaps the selectivity of the Na$^+$ pump in favour of Na$^+$ against Li$^+$ is not as high as has been thought[3,9].

The low levels of $[Li^+]_i$ accumulated with extracellular levels in the therapeutic range cast some doubt on a recent suggestion,

Fig. 4 Graph of the internal Li$^+$ concentration against time from an experiment in which a neurone was first exposed to a Ringer containing 45 mM Li$^+$, and then to one containing only 1 mM Li.



by Partridge and Thomas[7] that an increase in potassium conductance caused by intracellular Li$^+$ might be related to its psychiatric effects. It is perhaps possible that the 'Li$^+$ pump' varies in different neurones so that some might accumulate more Li$^+$ than others.

These results show that the new Li$^+$-sensitive microelectrode works well intracellularly, and suggest that it will be an important tool in future Li$^+$ research. It is clear that Li$^+$ ions are actively transported out of snail neurones, so that at low external levels there is even less Li$^+$ intracellularly than extracellularly. This makes it harder to see how Li$^+$ could act intracellularly to change neuronal properties in Li$^+$ therapy.

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Experimental evidence that polyoma-specific tumour antigen is a virus-coded protein

POLYOMA virus (PV) induces intranuclear tumour (T) antigen formation in permissive and semipermissive cells. In virus-infected cells, T antigen appears before the onset of viral DNA synthesis and is continuously demonstrable in PV-transformed cells[1]. The biological function of the T antigen is still uncertain, but the temporal relationship between early virus-specific messenger RNA (mRNA) synthesis, T antigen formation and stimulation of host cell DNA synthesis led to the hypothesis that T antigen acts as an activator for DNA synthesis[2,3]. Furthermore there exists only indirect evidence that T antigen is a virus-coded protein[1]. We have tested this by a more direct experimental approach. For this reason PV DNA was transcribed *in vitro*, and the complementary RNA (cRNA) obtained was further purified and injected into mouse tissue culture cells by our microinjection technique[4,5]. PV-specific T and V antigen formation was investigated.

PV DNA I was used as template for the *Escherichia coli* DNA-dependent RNA polymerase. After transcription viral DNA was thoroughly removed by exhaustive DNase treatment (M.G. and A.G., unpublished). To prevent any contamination with viral DNA, the cRNA preparation was then centrifuged to equilibrium in Cs_2SO_4 at a density excluding the entrance of PV DNA into the gradient (M.G. and A.G., unpublished and ref. 6) (Fig. 1).

The size of the cRNA was estimated by formaldehyde–sucrose gradient centrifugation. Figure 2 shows that more than 50% of the PV cRNA has a sedimentation coefficient of 26S or more. The complete transcription product of one PV DNA strand would have a molecular weight of 1.6×10^6, sedimenting as 26S RNA in these centrifugation conditions[8]. *E. coli* RNA polymerase transcribes both the early and the late strand of the non-defective wild-type PV DNA I[9]. Depending on the preparation 60–80% of the cRNA

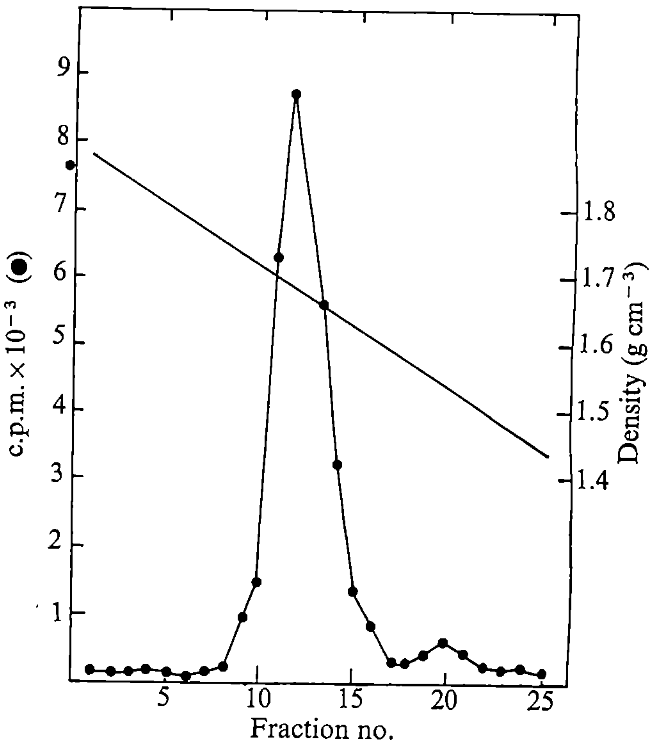


Fig. 1 Cs_2SO_4 equilibrium density gradient centrifugation of PV cRNA. Cultures of confluent primary mouse kidney cells were infected with plaque-purified (three times) wild-type virus (PV78) at a multiplicity of 0.1 PFU per cell. After an adsorption period of 2 h cells were washed and covered with serum-free Eagle's medium PV DNA was extracted by the extraction method of Hirt[7]. Purification of PV DNA involved RNase treatment (Worthington, 50 µg ml^{-1}, 30 min, 37 °C), neutral sucrose gradient centrifugation (5–20% w/v, 1 M NaCl, 0.001 M EDTA, 0.001 M Tris-HCl, *p*H 7.4) and CsCl–ethidium bromide equilibrium centrifugation (1 56 g ml^{-1} CsCl, 100 µg ml^{-1} ethidium bromide, 0.02 M Tris-HCl, *p*H 8.0)[5]. PV DNA I was transcribed with *E. coli* DNA-dependent RNA polymerase (Miles Laboratories, *E. coli* K12, grade II). The transcription reaction mixture (400 µl) contained 10 µg PV DNA I, 10 µg RNA polymerase, 0 15 M KCl, 0.04 M Tris-HCl, *p*H 8.0, 5% glycerol, 0.01 M $MgCl_2$, 1.5 mM 2-mercaptoethanol, 0.4 mM each of ATP, GTP, CTP, UTP and 0.01 mM 8-^{3}H-GTP (Amersham Buchler, 15 Ci mmol^{-1}). Nucleoside triphosphates were added after preincubation of PV DNA and RNA polymerase at room temperature. After 60 min incubation at 37 °C, 20 µg electrophoretically purified DNase (Worthington) was added to the reaction mixture and further incubated for 30 min at 37 °C. Then 0 1 × SSC and 5% SDS were added to a final concentration of 0.01 × SSC and 1% SDS. The resulting mixture was extracted twice with one volume phenolhydroxyquinoline (80% phenol in H_2O v/v and 1% hydroxyquinoline w/v). The cRNA preparation was then subjected to Cs_2SO_4 equilibrium density gradient centrifugation. The initial density of Cs_2SO_4 in a solution of EDTA-Tris (0.001 M EDTA, 0.01 M Tris-HCl, *p*H 7.2) was 1.62 g cm^{-3}. The gradient was centrifuged in a Spinco SW65 rotor at 45,000 r.p.m. for 48 h at 5 °C. The gradient was fractionated from the bottom of the tube and fractions 10–14 collected. Cs_2SO_4 was removed by passing the pooled fractions through a Sephadex G-25 column and by exhaustive dialysis[6]. The average yield of cRNA was 40–50 µg with a specific activity of $1–2 \times 10^4$ c.p.m. per µg RNA Control experiments were made with 10 µg CH_3-^{3}H-thymidine-labelled PV DNA (1.9×10^5 c.p.m.; $1 9 \times 10^4$ c.p.m per µg DNA) which were transcribed as stated above (without 8-^{3}H-GTP): *a*, after DNase treatment the cRNA preparation contained 4×10^3 c.p.m. in acid-precipitable material; *b*, after Cs_2SO_4 equilibrium density gradient centrifugation pooled fractions 10–14 contained less than 100 c.p.m in acid-precipitable material equivalent to 0 05% initial DNA.

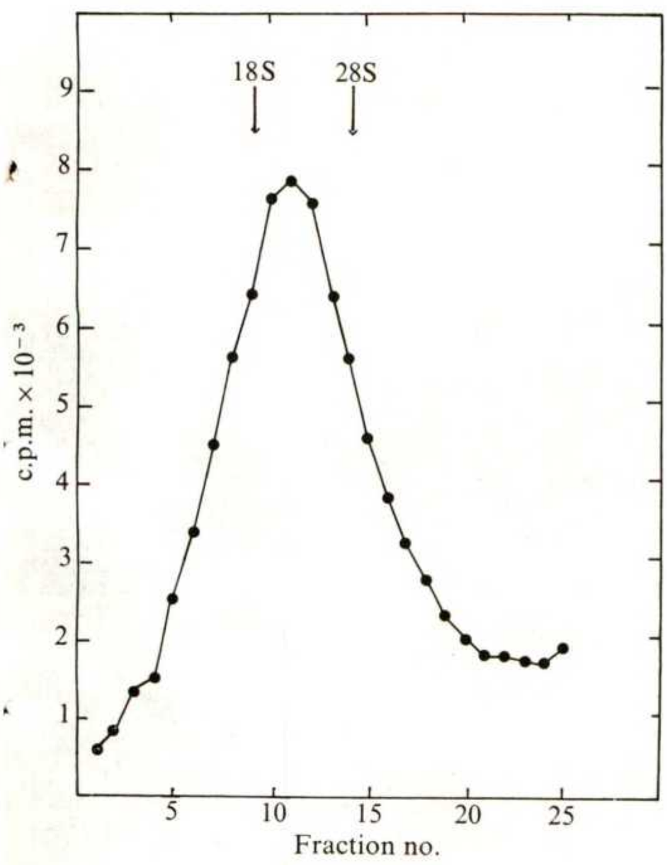
remained RNase resistant in self-annealing experiments (data not shown).

The biological activity of the cRNA was assayed in secondary mouse tissue culture cells after cRNA microinjection using our microinjection (glass capillary) technique[4,5]. The volume transferred into the cytoplasma of the recipient cells was about 10^{-11}–2×10^{11} ml per cell. After microinjection cells were further cultivated in Eagle's medium supplemented with 2% foetal bovine serum, fixed and stained for PV-specific T and V antigen at different hours after microinjection. Intranuclear T antigen formation was first detectable 6 h after cRNA transfer (Fig. 3). At this time about 6% of the recipient cells exhibited T antigen formation. After this time both the number of T antigen-positive cells as well as the intensity of T antigen-specific fluorescence per cell increased, reaching a maximum at 20–24 h. Thereafter both the proportion of T antigen-positive cells and the intensity of the T antigen-specific fluorescence decreased so that T antigen could not be detected 65 h after cRNA microinjection.

As shown in Table 1 the biological activity of the cRNA was destroyed by RNase treatment. Actinomycin D (5 μg ml^{-1}) inhibited T antigen formation in mouse cells either infected with wild-type PV (200 PFU per cell) or injected with PV DNA I (1 mg ml^{-1}), but did not even reduce cRNA-induced T antigen formation within the first 15 h. On microinjection neither total RNA nor polyadenylated RNA isolated from mouse cells induced T antigen formation.

PV cRNA preparation induced not only T but also V antigen (capsid protein) formation in microinjected secondary mouse cells. As shown in Fig. 3 the formation of V antigen on cRNA injection, although comparable in terms of time, was less efficient than the T antigen synthesis.

Fig. 2 Velocity sedimentation of PV cRNA in sucrose density gradient (7–27% sucrose w/v in high salt formaldehyde solution, 1.1 M HCHO, 0.09 M Na_2HPO_4, 0.001 M EDTA, pH 7.7). The gradient was centrifuged in a Spinco SW65 rotor at 49,000 r.p.m. for 3 h at 20 °C. Before centrifugation PV cRNA was heated for 5 min at 80 °C in low salt formaldehyde solution (1.1 M HCHO, 0.0045 M Na_2HPO_4, 0.0005 M NaH_2PO_4, 0.001 M EDTA, pH 7.7).



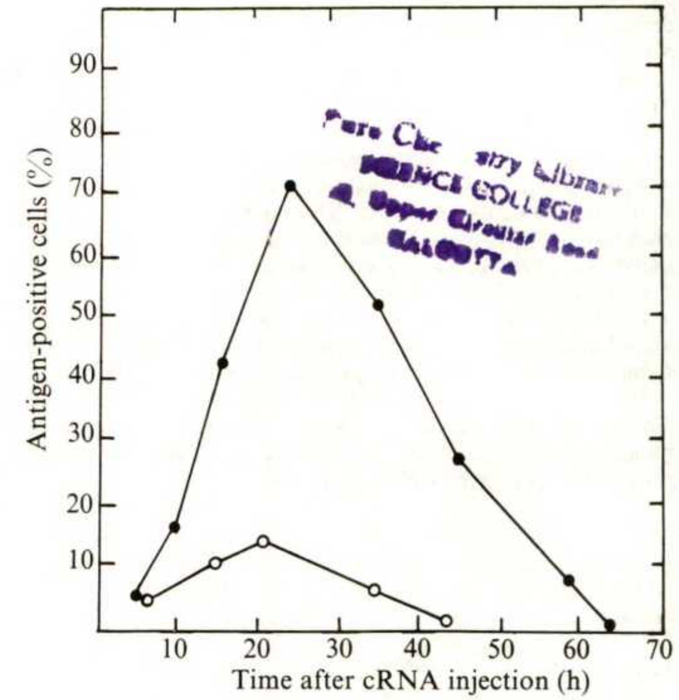


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Fig. 3 Time course of T and V antigen formation in secondary mouse tissue culture cells. Secondary mouse cells grown on glass slides (10 × 50 mm, subdivided in squares of 1 mm^2) to confluence were used as recipient cells. Transfer of cRNA was carried out under a phase-contrast microscope (×400) by help of micro glass capillaries having a diameter at the tip of about 0.5 µm. cRNA was dissolved in injection buffer (0.048 M K_2HPO_4, 0.014 M NaH_2PO_4, 0.0045 M KH_2PO_4, pH 7.2) at a concentration of 0.5 mg ml^{-1}. At the time indicated cells were fixed (acetone–methanol 3:2 v/v, for 10 min at −20 °C) and stained for T and V antigen by indirect immunofluorescence technique. Each time point is based on a count of 400 injected cells. ●, T antigen; ○, V antigen.

Our results demonstrate that the *in vitro* synthesis of PV cRNA, consisting of RNA complementary to both early and late viral DNA strand, induces PV-specific T and V antigen formation on microinjection into secondary mouse

Table 1 Polyoma virus T antigen formation in mouse cells

Injection of	Intranuclear T antigen formation (%)*
PV cRNA (0.5 mg ml^{-1})	42
PV cRNA (0.5 mg ml^{-1}) in actinomycin D-treated cells	43
PV DNA I (1 mg ml^{-1}) in actinomycin D-treated cells†	0
PV DNA I (0.01 mg ml^{-1})‡ + mouse cell RNA§ (1 mg ml^{-1}) in actinomycin D-treated cells†	0
PV cRNA (0.5 mg ml^{-1}), RNase-treated[4]	0
PV cRNA (0.5 mg ml^{-1}), alkaline-treated[4]	0
Mouse cell RNA§	0

*Each measurement is based on a count of 400 injected cells. Cells were fixed and stained 15 h after injection.

†Actinomycin D was added to a final concentration of 5 µg per ml medium 30 min before microinjection of nucleic acids. Cells remained in this medium during and after injection procedure until fixation.

‡Without actinomycin D this DNA concentration induces T antigen synthesis in 100% of injection cells.

§Total cellular RNA extracted from mouse kidney cells[4].

tissue culture cells. The fact that the cRNA preparation was, in addition to DNase treatment, further purified by Cs$_2$SO$_4$ equilibrium centrifugation indicates that the virus-specific reactions are not due to a small contamination of viral DNA. This is also supported by the observation that cRNA-induced T antigen formation is sensitive to RNase treatment and that actinomycin D prevented T antigen synthesis in PV DNA-injected cell but not the cRNA-induced T antigen formation. These data support the hypothesis that PV-specific T antigen is a virus-coded but not a cell-coded protein. Our results do not exclude the possibility, however, that T antigen is a protein consisting of multiple subunits, in part virus and in part cell coded, or that the cRNA translation product modifies a constitutive cellular compound which then reacts immunologically as T antigen.

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Correlation of polypeptide composition with antigenic variation in the swine vesicular disease and coxsackie B$_5$ viruses

SINCE the observation by Graves[1] that swine vesicular disease virus (SVDV)[2] is neutralised by antiserum to coxsackie B$_5$ (CB$_5$) virus, an enterovirus infecting man, and observations[3] that the swine virus can also infect man, there has been speculation about the relationship between the two viruses and the possible role of CB$_5$ virus in the aetiology of the swine disease[4]. Further work has shown that the two viruses can be differentiated by neutralisation tests and also that there has been considerable antigenic drift in the CB$_5$ viruses[5,6]. RNA hybridisation experiments have corroborated these antigenic relationships; there is approximately 50% homology between the RNA of the UK isolate of SVDV and the RNA of the prototype (Faulkner) CB$_5$ and there is a similar level of annealing between prototype CB$_5$RNA and the RNA of two recent isolates of CB$_5$ (8068 and 9954). These variations prompted an investigation into some of the other characteristics of SVDV and CB$_5$.

The sedimentation coefficient (S value) of several isolates of SVDV and CB$_5$ was determined and compared with that of poliovirus, the 'type' enterovirus[7], by sedimenting mixtures of two viruses labelled with either ^{35}S-methionine or ^{3}H-amino acids (produced and purified as described in the figure legends) through 15–45% (w/v) sucrose gradients. The S value of SVDV isolates (UK, France 1/73 and 2862) and CB$_5$ isolates (Faulkner and 8068) measured in this way were similar to each other and to polio virus, indicating that the S value of polio virus (160S)[8] can also be assigned to CB$_5$ and SVDV. Similar mixtures of purified viruses were used to compare the buoyant densities of SVDV and CB$_5$ in caesium chloride gradients ($\rho = 1.29$–1.42 g cm^{-3}) in 0.1 M NaCl, 0.05 M Tris–HCl, *p*H 7.6 by centrifuging at 75,000 *g* overnight at 20 °C (20,000 r.p.m., MSE 59108 rotor). All the SVDV isolates, CB$_5$ (8068) and polio virus came to equilibrium at a density of 1.34–1.35 g cm^{-3} whereas the prototype CB$_5$ (Faulkner) had the slightly higher buoyant density of 1.35–1.36 g cm^{-3}. Buoyant density measurements have been used as a criterion for classifying mammalian picornaviruses and the values obtained here agree with those reported for other enteroviruses[8]. The slightly higher value obtained for prototype CB$_5$ is interesting and may

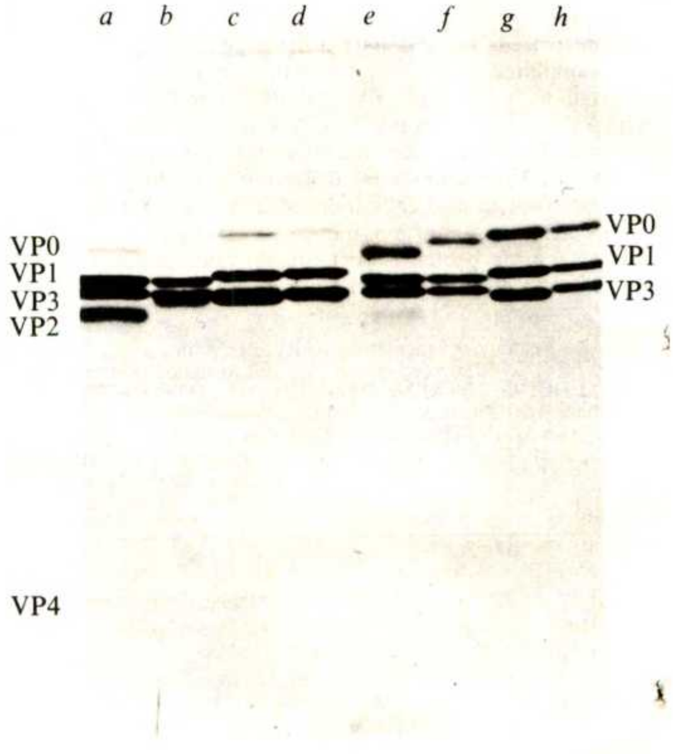

Fig. 1 Autoradiograph of a polyacrylamide gel slab of the polypeptides of ^{14}C-labelled full and empty particles of SVDV and CB$_5$ virus. Full particles of: *a*, SVDV (UK); *b*, SVDV (HK 71); *c*, SVDV (France 1/73); *d*, CB$_5$ (8068); *e*, empty particles of SVDV (UK); *f*, SVDV (HK 71); *g*, SVDV (France 1/73); *h*, CB$_5$ (8068). The viruses were grown in IBRS-2 cell monolayers[15] labelled with ^{14}C-amino-acids (5–10 μCi ml^{-1}, 45 μCi per m atom carbon, Radiochemical Centre) in 20 ml Earle's saline, 1.5 h after high multiplicity infection. They were purified from this medium after overnight incubation at 37 °C[16]. The 18-ml sucrose gradients (15–45% (w/v) sucrose in 0.1 M NaCl, 0.05M Tris-HCl, *p*H 7.6 at 25 °C) were centrifuged at 80,000*g* for 2.25 h at 10 °C (30,000 r.p.m. MSE SW.25). The peaks of virus or empty particles were pooled, diluted with 0.1 M NaCl, 0.05 M Tris-HCl containing 75 μg bovine plasma albumin and protein precipitated with 2 volumes of acetone at −20 °C overnight. The precipitate was deposited by centrifugation, dried, resuspended in disruption buffer (0.0625 M Tris-HCl, *p*H 6.8 containing 0.5 M urea, 2% (w/v) SDS, 0.2% β-mercaptoethanol and 10% (v/v) glycerol) and analysed on high resolution acrylamide gel slabs[17] using Tris-glycine buffer[14]. The main gel (12.5%) and the stacking gel (5%), modified to contain 0.5 M urea, were subjected to electrophoresis for 4–6 h at 100 V. The gel was stained with 0.25% (w/v) Coomassie brilliant blue in methanol–acetic acid (2:1), destained in methanol–acetic acid, dried under vacuum on to wettable Cellophane and autoradiographed with Kodak RP Royal X-Omat or Industrex X-ray film for 5–10 d.

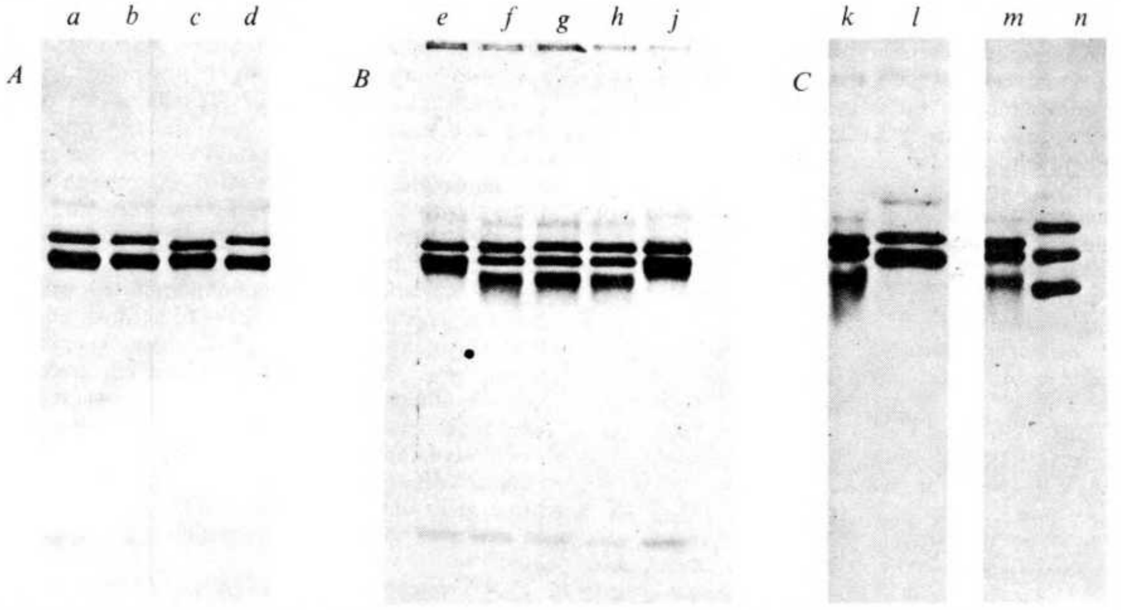

Fig. 2 Autoradiographs of polyacrylamide gel slabs of the polypeptides of several ^{35}S-methionine-labelled CB_5 and SVDV isolates. *A*, CB_5 isolates: *a*, 8068; *b*, 9954; *c*, Faulkner; *d*, HKB_5; *B*, SVDV isolates: *e*, Italy 1/66; *f*, UK; *g*, Austria 1973; *h*, Italy 1972; *j*, Hong Kong, 1971; *C*, SVDV isolates: *k*, UK; *l*, France 1/73; *m*, 2862; *n*, polio virus, type 1 (strain Mahoney). The viruses were grown, purified and prepared for electrophoresis as described in the legend to Fig. 1 but were labelled with ^{35}S-methionine (2–5 μCi ml^{-1}, 100 Ci mmol^{-1}, Radiochemical Centre) in 20 ml of methionine-free Eagle's medium. ^{35}S-polio virus was grown in HeLa cells in a similar way. The gels were subjected to electrophoresis and autoradiography as described in Fig. 1.

reflect some slight variation in the fine architecture of the virus particle of this strain.

The antigenic diversity between SVDV and CB_5 is presumably a reflection of some difference in the proteins of the virus particle. It seemed possible, therefore, that the pattern of polypeptides obtained by polyacrylamide gel electrophoresis of reduced and sodium dodecyl sulphate (SDS) disrupted virus particles might be distinct and perhaps diagnostic of a particular isolate. Comparison of the polypeptides of several purified isolates of SVDV and CB_5 by electrophoresis in 12.5% slab gels using a continuous buffer system at neutral *p*H (ref. 10), however, gave poorly resolved polypeptide patterns which could not be distinguished reproducibly. The patterns produced were similar to those published previously for selected strains of SVDV and CB_5 (refs 11 and 12) using a similar buffer system, but unlike that obtained by Delagneau *et al.*[13] for the France 1/73 isolate of SVDV.

Well defined and reproducible differences in the mobility of the polypeptides were obtained, however, when a discontinuous Tris–glycine buffer system was used[14] (Fig. 1). The most variable polypeptides in terms of mobility were VPO and VP2. Polypeptide VPO, the precursor of VP2 and VP4[18], is found in empty particles and is also present in small amounts in the full particles of some picornaviruses[19,20]. The mobility of polypeptide VP2, which was identified by its virtual absence from empty particles (compare Fig. 1*a* and *e*), also varied. This is clearly demonstrated by the patterns in Fig. 1*a*–*d*. Whereas the mobility of VP2 in SVDV (UK) (Fig. 1*a*) is clearly different from that of VP3, these polypeptides are not well separated in SVDV Hong Kong 1971 and France 1/73 (Fig. 1*b*–*c*). (Comparison of the separations obtained with the polypeptides of the full particles of SVDV (HK 71) and France 1/73 in Fig. 1*b* and *c*, with those obtained with the polypeptides of the corresponding empty particles in Fig. 1*f* and *g* shows that the lower intense band in the full particle patterns consists of two polypeptides VP2 and VP3.) The variation was reproducible and occurred whether the viruses were labelled with ^{14}C-amino acids or ^{35}S-methionine (compare Figs 1 and 2). Little variation was found in the mobility of VP4 (Fig. 2).

The patterns obtained for the four CB_5 viruses (Fig. 2*A*) were clearly different from those of several of the SVDV isolates examined (Fig. 2*B* and *C*) as might have been expected from the clear cut differences found by neutralisation and immunodiffusion tests and by the RNA homology experiments[5,6]. The patterns for the three recent CB_5 isolates (8068 and 9954 from Birmingham, England and HKB_5 from Hong Kong (Fig. 2*A*, *a*, *b* and *d*)) were identical but differed from that obtained for the Faulkner strain (Fig. 2*A*, *c*), again in agreement with the neutralisation and immunodiffusion data.

Of greater interest was the difference between isolates of SVDV which showed only small and probably insignificant differences in neutralisation tests[21] and which had only been distinguished previously by the production of faint spur lines in immunodiffusion experiments[3,5] and by rather less than complete annealing in RNA hybridisation experiments[5]. The patterns obtained with isolates from outbreaks in Italy in 1966 (Fig. 2*B*, *e*) and in Hong Kong in 1971 (Fig. 2*B*, *j*) were clearly different from those obtained with viruses isolated in England, Austria and Italy in the winter of 1972–73 (Fig. 2*B*, *f*, *g* and *h*). These data suggest that the viruses causing the 1966 and 1971 outbreaks and those causing the disease in Europe in 1972–73 had a different origin, and that the European outbreaks may have been caused by a virus from a common source.

An interesting observation made during the examination of the SVDV isolates was the difference between the polypeptide patterns of France 1/73 virus (Fig. 2*C*, *l*), isolated in the Bordeaux area in January 1973 and those of the viruses in other European countries at about that time (Fig. 2*B*, *f*, *g* and *h*). Immunodiffusion tests[4] have shown that the France 1/73 isolate differed markedly from the other contemporary European isolates. It is also interesting that another isolate of SVDV (2862) (Fig. 2*C*, *m*) obtained from Annecy in the Haute Savoie area of eastern France at about the same time, gave a polypeptide pattern indistinguishable from that of the other European strains and also gave a line of identity with SVDV (UK) in immunodiffusion tests. Although the France 1/73 isolate of SVDV has a polypeptide pattern more like that of the recent CB_5 isolates, this need not imply that it is a CB_5 rather than an SVDV, but it emphasises the inherent variation in these viruses, the extent of which will only become clear after examination of more strains. Further detailed biochemical analysis of this variation may help to establish more exactly the relationship of SVDV to the CB_5 viruses and give some insight into the evolution of the swine viruses.

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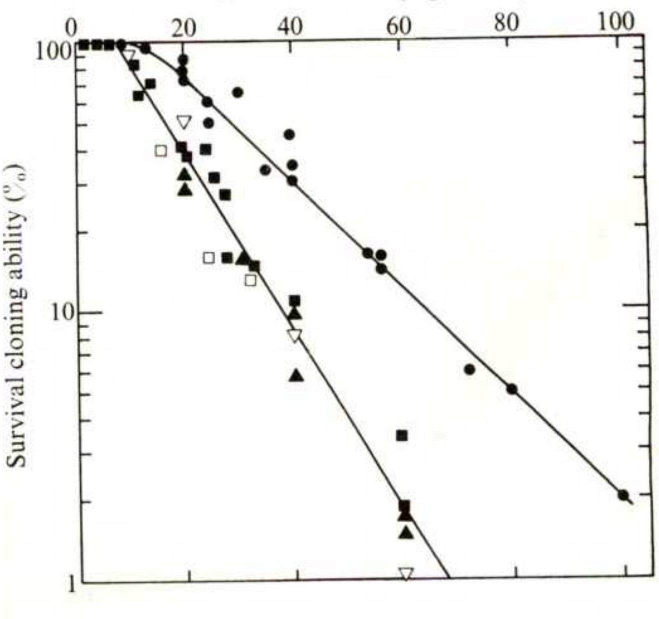
Synergistic effect of caffeine on the cytotoxicity of ultraviolet irradiation and of hydrocarbon epoxides in strains of Xeroderma pigmentosum

XERODERMA pigmentosum (XP) is an inherited human disease clinically characterised by abnormal pigmentation patterns and the development of multiple skin carcinoma on areas exposed to the Sun[1]. Cells from most XP patients are defective in excision repair of ultraviolet-induced lesions in DNA[1–3], but there is a class of patients—XP variants—who have all the clinical manifestations of the disease but who, until recently, appeared to have normal DNA repair. Cleaver[4] and others[5,6] have shown that variants have normal rates of excision repair of damage caused by ultraviolet light as measured by ultraviolet-stimulated uptake of labelled thymidine or bromodeoxyuridine. Cleaver also reported that variants XP4BE (his patient 13) and XP13BE (his patient 14) have normal sensitivity to ultraviolet light as measured by survival of colony-forming ability[4]. His survival data, however, consisted of only two points for each strain. As Fig. 1 shows, we find that the percentage survival of the colony-forming ability of variants XP4BE and XP13BE as well as variant XP7TA and XP30RO is more sensitive to ultraviolet irradiation than that of normal cells. We also showed that the survival of the cloning ability of XP variants is more sensitive than normal to active derivatives of four aromatic amide carcinogens[7] and to the K-region epoxides of three polycyclic hydrocarbons[8]. These two classes of carcinogens bind covalently to DNA[9,10] and the bound adducts seem to be recognised by the same repair enzymes which function in the repair of ultraviolet-induced damage in these human cells[7–11].

Preliminary evidence, from alkaline sucrose gradient studies, that such XP variants suffer from a defect in their DNA synthesis following ultraviolet irradiation was reported[12] and documented[6] by Lehmann and his coworkers. They showed that of three variants tested after irradiation, all were abnormally slow in converting initially low molecular weight DNA into high molecular weight DNA similar in size to that produced in irradiated cells. Other investigators[13] who reported that variant XP4BE (their patient WY-XP3), was normal in this gap-filling process had allowed 8 h for the process—too long to be able to distinguish the difference between XP variants and normal human cells. Lehmann *et al.*[6,12] also reported that in the XP variants this gap-filling process was completely inhibited by 1.5 mM caffeine while that of normal cells was insensitive to caffeine. This process is thought to be involved in survival of cells which have replicated DNA containing unexcised thymine dimers which distort DNA and have introduced gaps in the newly synthesised DNA[6]. We, therefore, wanted to determine whether caffeine, in non-toxic or only slightly toxic concentrations, would affect the survival of XP variants but not normal human cells after exposure to ultraviolet irradiation or to carcinogens which form lesions in DNA which may be repaired by the same processes used for dimers produced by ultraviolet light.

First, we compared the sensitivity of the different strains to caffeine itself. Figure 2 shows that the colony-forming ability of two XP variants (XP4BE and XP13BE) is significantly more sensitive to caffeine than that of normal cells. To determine whether this increased sensitivity was characteristic only of XP variants, we tested the survival of the colony-forming ability of strains derived from three classical XP patients—each from a different complementation group[1], namely, XP12BE from group A, XP2BE from group C, and XP6BE from group D. The percentage survival of these strains in the presence of caffeine is also depicted in Fig. 2. The colony-forming ability of

Fig. 1 Percentage survival, as a function of ultraviolet dose, of the colony-forming ability of normal strains of human skin fibroblasts (●) and that of four XP variant strains: ■, XP4BE; ▲, XP13BE; ▽, XP7TA; □, XP30RO. Survival of the cloning efficiency of irradiated cells divided by that of unirradiated controls is expressed as percentage. Cloning efficiencies of controls ranged from 15 to 35% for normal cells, from 10 to 25% for XP4BE and XP30RO and from 5 to 10% for XP7TA. The lower cloning efficiencies of this latter strain reflects the fact that it was available for testing only at a late passage number (> 30). The exponential portion of the curves were drawn by the least squares method. Each symbol represents the percentage survival averaged from a series of 8–12 replicate dishes of cells receiving the specified dose of ultraviolet irradiation. Variation in the number of colonies per dish was smaller than the size of symbol used. Procedures for cytotoxicity cloning experiments have been described elsewhere[7,14].



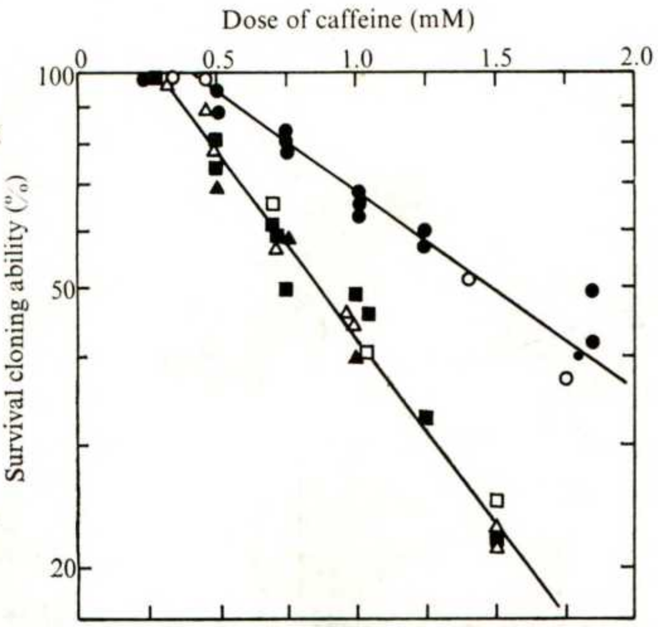


Fig. 2 Effect of caffeine on the cloning ability of normal cell (●), XP2BE (○), XP6BE (□), XP12BE (△), XP4BE (■), XP13BE (▲). From 250 to 6,000 cells in F10 medium (Ham's) containing 15% foetal bovine serum were plated in 60-mm dishes, allowed 8–10 h to attach and then fed medium containing the designated concentrations of caffeine three times weekly for 7–10 d and then caffeine-free medium until macroscopic colonies developed and could be stained. Survival of cloning ability was plotted as in Fig. 1.

XP12BE from complementation group A and XP6BE from group D proved to be just as sensitive to caffeine as the two XP variants, while the other classical XP strain resembled the normal cells in sensitivity to caffeine.

We then compared the effect of caffeine on the survival of the cloning ability of these strains after exposure to such DNA-damaging agents as ultraviolet irradiation or hydrocarbon epoxides. Cells were plated, exposed to the particular agent, and then left to form macroscopic colonies in the presence or absence of the specified concentrations of caffeine. The cytotoxic effect of each concentration of caffeine itself was determined from the untreated control dishes exposed to that particular dose. The effect of caffeine on the ultraviolet survival curves of normal cells, XP variants and classical XP strains is shown in

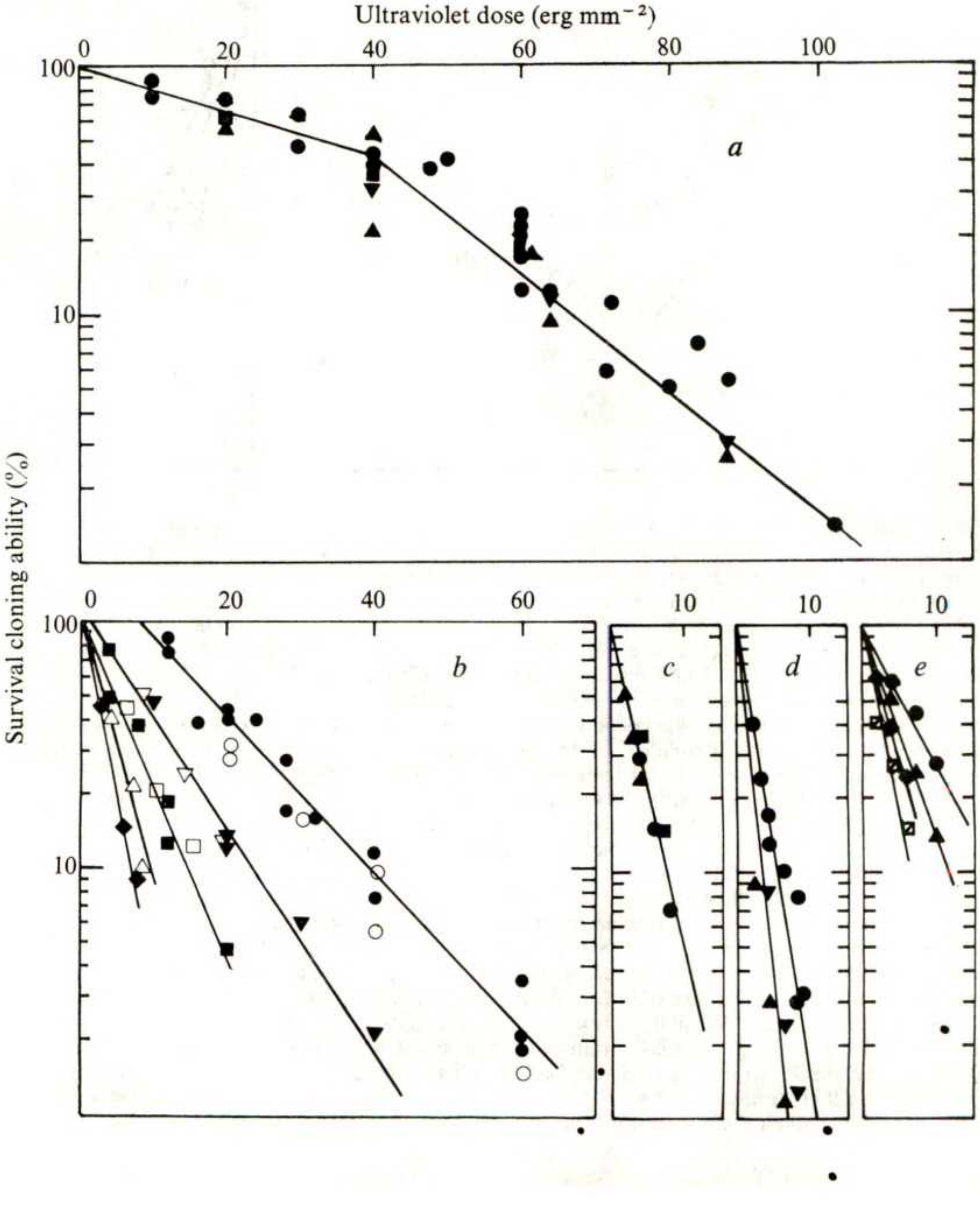


Fig. 3 Effect of caffeine on the cytotoxicity of ultraviolet irradiation in the various strains. Cells were plated, allowed to attach, irradiated as described previously[7] with the specified doses of ultraviolet irradiation and fed medium containing the specified concentrations of caffeine three times weekly for 7–10 d and then caffeine-free medium until macroscopic clones developed. For each concentration of caffeine, the survival of the cloning abilities of the particular strains are plotted as percentage of cloning efficiency of its unirradiated control to correct for any killing by that particular concentration of caffeine. Cells were protected from light during all operations. *a*, Normal fibroblasts; *b*, XP4BE (closed symbols); and XP13BE (open symbols); *c*, XP6; *d*, XP12; *e*, XP2. Concentrations of caffeine (mM): ●, 0.0; ▼, 0.5; ■, 0.75; ▲, 1.0; diamond, 1.5; square and and horizontal bar, 1.75.

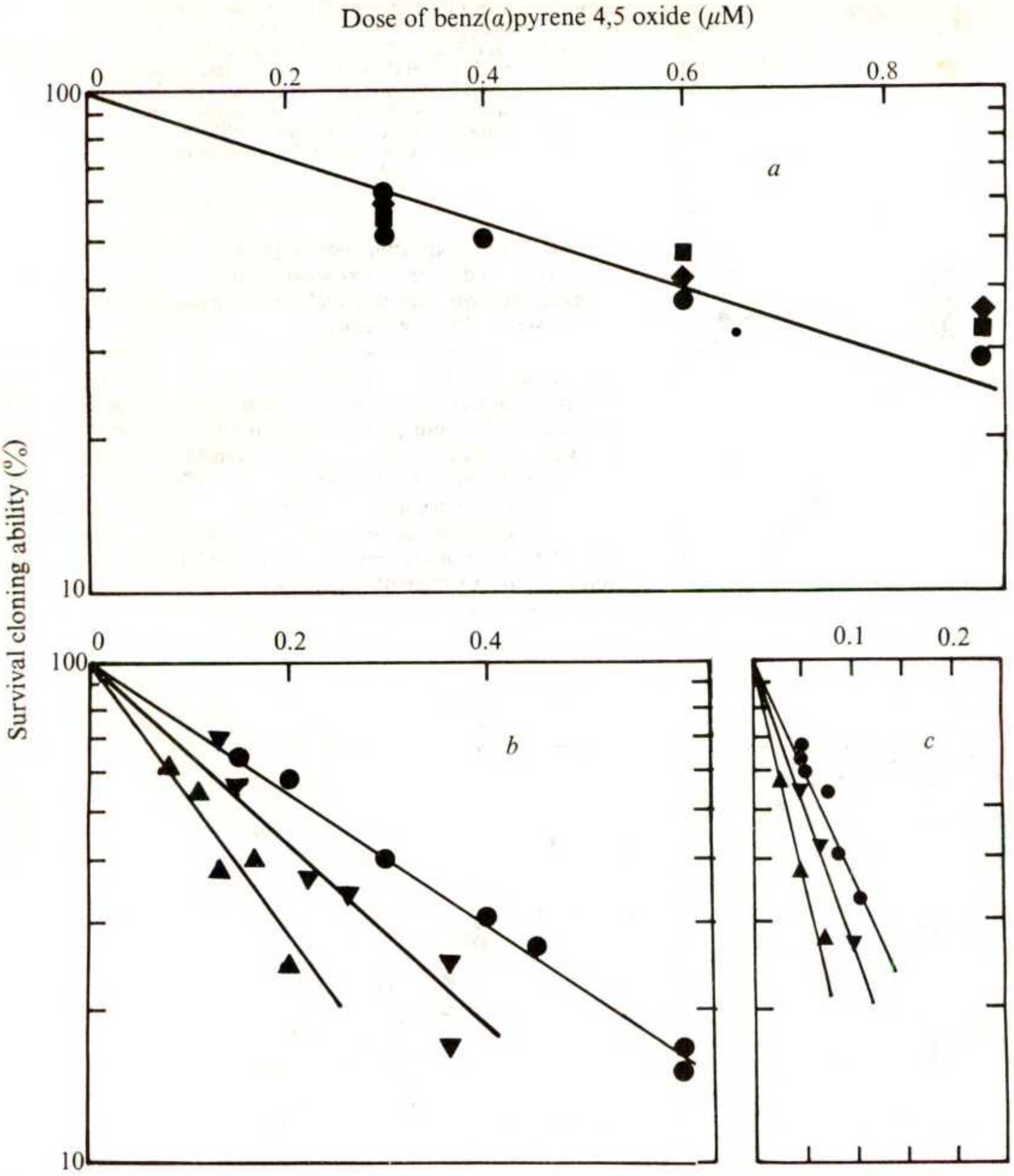


Fig. 4 Effect of caffeine on the cytotoxicity of benz (α) pyrene 4,5 oxide in the various strains. Percentage survival of the cloning ability at each concentration of caffeine was plotted as in Fig. 3. Detailed procedures for the exposure of cells to freshly prepared anhydrous acetone solutions of the carcinogen have been described[7,8]. *a*, normal fibroblasts; *b*, XP4BE; *c*, XP12. Symbols represent concentrations of caffeine as in Fig. 3.

Fig. 3. Each survival curve was corrected for any cell killing caused by exposure to the caffeine alone. Therefore, an increased slope in a survival curve indicates that the presence of caffeine during the repair period has potentiated the killing effect of the ultraviolet exposure, that is, it has had a synergistic effect on the cytotoxicity of ultraviolet irradiation. The data show that even in a dose as low as 0.75 mM, caffeine increases the cytotoxicity of ultraviolet irradiation in the variant strains, XP4BE and XP13BE, and that this synergistic effect increases with increasing concentrations of caffeine. In contrast, caffeine, at comparable cytotoxic concentrations (compare Fig. 2), causes no measurable increase in the slope of the ultraviolet survival curve of the normal cells or XP6BE from group D and, at most, exerts a slight synergistic effect on the classical XP strains, XP12BE group A and XP2BE group C.

The rate of the gap-filling process in a classical XP from group A and from group C, as measured by physical means, is reported[6] to be intermediate between that of normal cells and XP variants and to be intermediately sensitive to caffeine. This may account for the slight synergistic effect of caffeine observed in these biological studies. We find, however, no direct correlation between the sensitivity of a cell strain to caffeine and the ability of caffeine to exert a synergistic effect on ultraviolet cytotoxicity in that strain.

We have shown that ratios of the slopes of the survival curves of XP variants and two classical XP strains to that of normal cells after exposure to hydrocarbon epoxides resemble those found for ultraviolet irradiation[7]. This suggests that these cells repair at least some of the DNA damage caused by the hydrocarbon treatment by processes similar to those used to repair ultraviolet damage. We therefore compared the effect of caffeine on the survival of such strains after exposure to the K-region epoxide of benz(α)pyrene (Fig. 4). It is evident that caffeine, even in cytotoxic doses, has no potentiating effect on the cytotoxicity of this compound in the normal cells; exerts a dose-dependent synergistic effect on the killing by the epoxide in the XP variant, and has an intermediate effect in the classical XP from group A. These results support the hypothesis that the cellular DNA repair processes involved in handling such DNA-damaging agents are similar to those used for ultraviolet induced lesions. We are examining these strains of human cells for the effect of caffeine on cell survival after exposure to other types of DNA-damaging agents, which induce ultraviolet-like repair, such as aromatic amide carcinogens.

Cleaver has shown that caffeine does not inhibit unscheduled DNA synthesis in HeLa cells[15]. Our survival curves for the normal fibroblasts support this conclusion. Since the XP variants have been shown by several methods to have normal excision repair[4–6], one would not expect caffeine to inhibit excision in these strains. It seems to interfere with the gap-filling process[6] and it renders the cloning capacity of these cells more sensitive than normal to DNA-damaging agents. The mechanism by which caffeine causes the observed effects in the XP strains is unknown. But survival studies such as ours are important because they demonstrate that a defective repair process, previously identified by physical techniques, manifests itself directly in a reduction in cell survival. Our data with these XP variant strains provide evidence, in addition to that of Day[16], for a relationship between defective DNA repair and the clinical manifestation of XP.

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Cyclic AMP-induced changes in protein synthesis in a cellular slime mould, *Polysphondylium pallidum*

IN addition to acting as a chemotactic signal[1,2], cyclic AMP induces the formation of stalk cells from undifferentiated amoebae in the cellular slime mould *Dictyostelium*[3,4]. The inductive action is specific for cyclic AMP, as AMP, ATP, ADP, GMP and GTP have no effect[3]. The induction also acts on *Polysphondylium pallidum*, a related species, even though cyclic AMP is not chemotactic for this species[5,6].

There are two hypotheses at present for the general mechanism of cell differentiation in these organisms: Sussman[7] has proposed that a succession of new genes becomes active and that the resulting succession of newly synthesised enzymes causes the morphological changes of cell differentiation. This agrees with the known effects of cyclic AMP on transcription of new genes, both in higher eukaryotes[8–10] and in bacteria[11,12]. Wright and colleagues[13,14] argue that the causes of visible development are primarily a succession of changing substrate concentrations, interacting with a relatively stable complement of enzymes. Newly synthesised proteins are not the primary "critical variables" by this theory. Rosness and Wright[15] have suggested that cyclic AMP might act on cell differentiation by directly affecting the conversion of glycogen to cellulose in stalk cells and spores.

In this paper, acrylamide gel electrophoresis and auto-

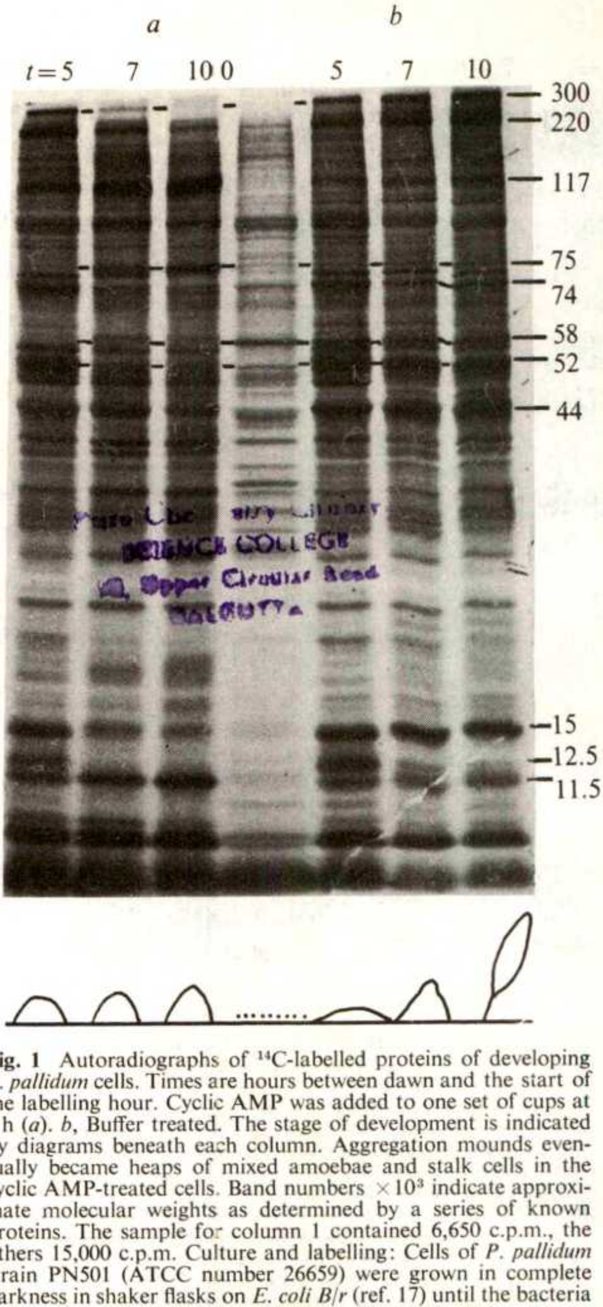

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Fig. 1 Autoradiographs of ^{14}C-labelled proteins of developing *P. pallidum* cells. Times are hours between dawn and the start of the labelling hour. Cyclic AMP was added to one set of cups at 2 h (*a*). *b*, Buffer treated. The stage of development is indicated by diagrams beneath each column. Aggregation mounds eventually became heaps of mixed amoebae and stalk cells in the cyclic AMP-treated cells. Band numbers $\times 10^3$ indicate approximate molecular weights as determined by a series of known proteins. The sample for column 1 contained 6,650 c.p.m., the others 15,000 c.p.m. Culture and labelling: Cells of *P. pallidum* strain PN501 (ATCC number 26659) were grown in complete darkness in shaker flasks on *E. coli B/r* (ref. 17) until the bacteria were gone and the amoebae began to clump. The cultures were centrifuged without cooling and the pellet resuspended in 0.017 M phosphate buffer, *p*H 6.0. The cells were dispensed on to a dialysis membrane stretched over one end of a 0.5-inch diameter plastic cylinder (a 'cup'), at a density of 5×10^6 cells per cup (corresponding to 240 μg protein per cup and to 4.5×10^4 cells mm^{-2}). These operations were carried out under a sodium-vapour darkroom safelight emitting light of 589 nm, a wavelength to which this strain is very slightly sensitive[18]. Development was initiated by exposing the cups to fluorescent light of intensity 138 lx. At the same time the cups were inverted over a layer of charcoal, which absorbed volatile inhibitors of development, and the dialysis membrane was covered with a buffer-soaked filter disk to prevent drying. To label proteins, the filter disk was replaced with a Millipore filter saturated with 25 μl buffer or with buffer with 5×10^3 M cyclic AMP added, plus 10 μC per pad of *l*-^{14}C sodium acetate. The total acetate concentration was 7×10^3 M. Cells were collected after one hour of labelling by brushing them into 100 μl sample buffer[19] added to the cup. Samples were stored at −90 °C. Electrophoresis: Samples of dissolved cells were thawed, then placed in boiling water for 1 min to dissolve proteins. In general, samples were diluted so that 25 μl had 10,000–50,000 c.p.m. TCA-precipitable material; this amount was used for electrophoresis. Where practical the samples run on a single gel were diluted to equal c.p.m. Discontinuous SDS electrophoresis in 10% polyacrylamide slabs was by a modification of the procedure of Laemmli[19,20]. After electrophoresis, the gels were stained with Coomassie blue, dried and autoradiographed using Kodak X-ray film RP/R54, developed with Diafine developer.

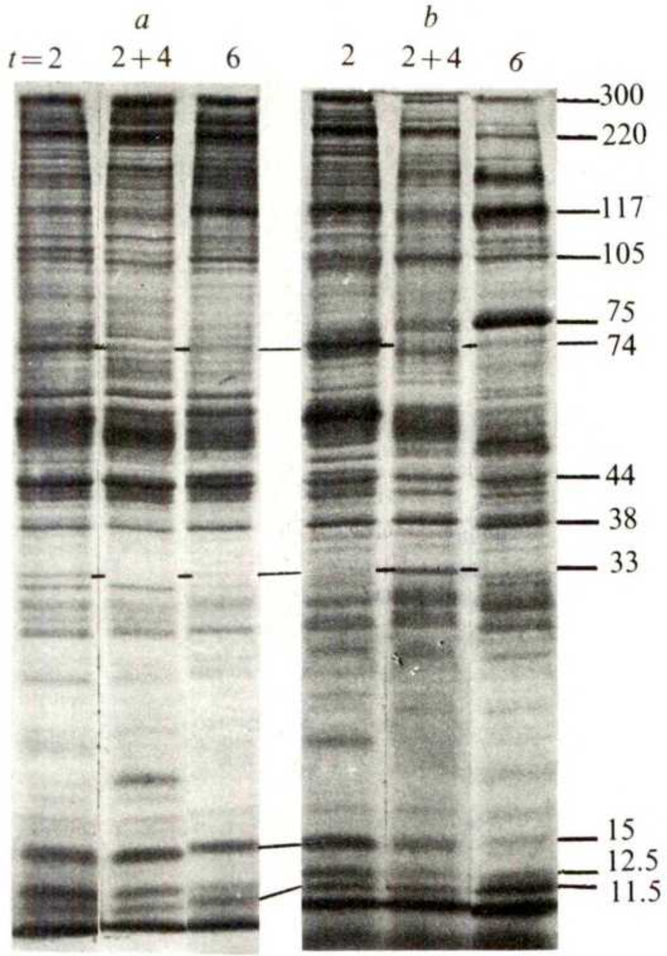

Fig. 2 Autoradiographs of pulse-chase experiment. Here cyclic AMP was added to the experimental set at dawn ($t = 0$). Cups labelled at $t = 2$ h were collected either at the end of the 1-h labelling period (first and fourth columns) or after a 4-h chase period (second and fifth columns). To chase, the Millipore disk containing labelled acetate at 1×10^{-3} M was replaced by a filter paper disk of cold acetate at 7×10^{-3} M. Comparison of changes in individual band intensities in columns 1 and 2 with those in columns 4 and 5 indicates the degree to which cyclic AMP affects proteolysis. Band 33 increases in intensity during the chase period in cyclic AMP-treated cells, and may represent a subunit created by induced proteolysis of a larger protein. *a*, Buffer treated; *b*, cyclic AMP-treated.

radiography were used to compare synthesis of specific proteins in normally developing and in cyclic AMP-treated cells. The results indicate that induction of stalk cells by cyclic AMP necessarily involves alterations in net synthesis of several proteins.

Dark-grown cells of *P. pallidum* were dispensed on to dialysis membranes stretched over small cups. Development was initiated by exposing the cups to light. Two hours after dawn development was in mid-aggregation, and some of the cups were exposed to 5 mM cyclic AMP. To detect protein synthesis, cells were labelled with ^{14}C-acetate and collected at intervals from time zero (dawn) to 10 h (the early sorogen stage in the untreated cups). Control cups were inspected on the following day for presence or absence of heaps of mature stalk cells. The cell samples were used for measurement of trichloroacetic acid (TCA)-precipitable material and for sodium dodecyl sulphate (SDS)–acrylamide electrophoresis.

An immediate effect of cyclic AMP exposure was a temporary decrease in the rate of precursor incorporation into TCA-precipitable material by approximately 30%. In addition cyclic AMP induced differences in labelling between normally developing and treated cells in about 10 of the approximately 55 resolvable bands shown in Fig. 1. Roughly half of the changes consist of the disappearance of a band in the cyclic AMP-treated cells, whereas the same band remains steady in the normally developing controls (for example, numbers 300, 220, 74, 58 and 15). In three cases, a band becomes denser in the cyclic AMP-treated cells and remains steady or fades in the control cells (number 117, 75 and 11.5). In at least one case (12.5) a band which changes during normal development changes in a similar fashion in cyclic AMP-treated cells. A large majority of bands are steady in both wild type and cyclic AMP.

In every case except one, the changes are in development-correlated bands—that is, bands which change in one way or another during normal development between time zero when the lights are turned on and 10 h. These observations indicate that the effect of cyclic AMP is not to affect proteolysis or protein synthesis in general, but is highly specific.

To distinguish between changed synthesis and changed degradation as causes of altered protein net synthesis, cells were pulse labelled with ^{14}C-acetate for 1 h, then chased with cold acetate for 4 h. The results (Fig. 2) indicate that fading of certain bands following cyclic AMP treatment is accelerated (numbers 300, 220, 15 and 12.5), whereas others remain relatively constant (numbers 38, 44 and 105). This indicates that cyclic AMP induces specific proteolysis. In the case of two bands which increase in intensity following cyclic AMP treatment (numbers 11.5 and 75) no proteolysis is detectable with or without cyclic AMP, which suggests that cyclic AMP induces new synthesis of these proteins.

Bonner[3] has reported that stalk cell induction by cyclic AMP occurred even in the presence of cycloheximide, which (if so) would argue against the notion that new protein synthesis is essential for stalk cell induction. When cycloheximide (200 μg ml^{-1}) was added to cells of *P. pallidum* at the same time as cyclic AMP, total protein synthesis dropped to 21% of control, and synthesis of most proteins, including all of those affected by cyclic AMP, was greatly reduced. These amoebae did not differentiate to stalk cells. This contradiction of Bonner's finding remains unexplained.

In other circumstances in which cyclic AMP does not induce stalk cells the specific band changes do not occur. Cells kept in constant darkness, for example, failed to aggregate and did not respond to cyclic AMP addition by either specific band changes or subsequent stalk cell differentiation. Similarly, cells which had embarked on formation of microcysts and exhibited a corresponding set of protein changes different from those of aggregation, also showed no response to cyclic AMP.

The fact that precursor incorporation into protein bands shows specific increases or specific decreases following cyclic AMP treatment, together with the fact that these changes are always correlated with the induction of stalk cells and do not occur when stalk cells are not induced, argues strongly that cyclic AMP exerts its inductive effect on cell differentiation by affecting synthesis and breakdown of particular proteins. To this extent the results support the hypothesis of Sussman. Note, however, that while these changes in protein synthesis are occurring, the cells are still amoeboid. The morphological changes of increased vacuolisation and formation of cell wall polysaccharides which result in actual stalk cells[16] do not occur until 10–20 h later. It remains possible that the temporally immediate causes of these morphological changes are fluctuations in substrate concentrations, as proposed by Wright. A general theory of development might include elements from both hypotheses.

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Actin polymerisation induced by spectrin

EUKARYOTIC cells are thought to contain a framework of fibrillar proteins—or 'cytoskeleton'—which is a basis for their shapes and movements. To understand this structure it is necessary to know both the properties of the component proteins and the way they are linked together. Actin and spectrin are two such structural proteins: actin is familiar for its part in muscle contraction, but is present partly as microfilaments[1,2] throughout the cytoplasm of most cells; spectrin is the major protein of red blood cell membranes and makes up a network on the cytoplasmic surface[3,4].

We were led to study the interaction of spectrin and actin by the observation that endogenous membrane-associated actin copurified with the spectrin of human erythrocytes. Spectrin is extracted together with small amounts of protein of lower molecular weight from erythrocyte ghosts in media of low ionic strength. When purified by column chromatography on Sephadex G–200, the spectrin emerges at the void volume and most of the other protein elutes later. Spectrin so isolated is pure, as judged by gel electrophoresis in the presence of SDS, except that a protein which migrates at the same position as muscle actin is always present. This corresponds to membrane component 5 in the nomenclature of Fairbanks *et al.*[5], and evidence that it is like actin in other respects has been mentioned[6]. For the experiments described below spectrin was prepared by extraction of washed ghosts with water for 1 h at 37 °C; it was purified by chromatography on a column (2.2×50 cm) of Sephadex G–200, and concentrated when necessary by vacuum dialysis. Rabbit muscle actin was prepared according to the method of Spudich and Watt[8]. At low ionic strength muscle G-actin remains in a monomeric state (molecular weight 42,000), and does not polymerise, so that on a Sephadex G–200 column in these conditions it should readily separate from spectrin. We observed, however, that nearly all the protein in an actin–spectrin mixture emerged in a single peak which showed both components on SDS–acrylamide gels.

To establish whether actin had bound to spectrin to form aggregates, or had been induced to polymerise into filaments, we measured the viscosity of actin–spectrin mixtures. In this low ionic strength solution, actin or spectrin alone had low and constant viscosities, but on mixing, the viscosity rose sharply in a manner characteristic of the formation of long polymers (Fig. 1). The same results were obtained when EDTA was present to eliminate calcium and magnesium ions. The final viscosity reached was similar to that shown by actin alone in the presence of 0.1 M KCl; if KCl was added to the actin–spectrin mixture no further increase was observed.

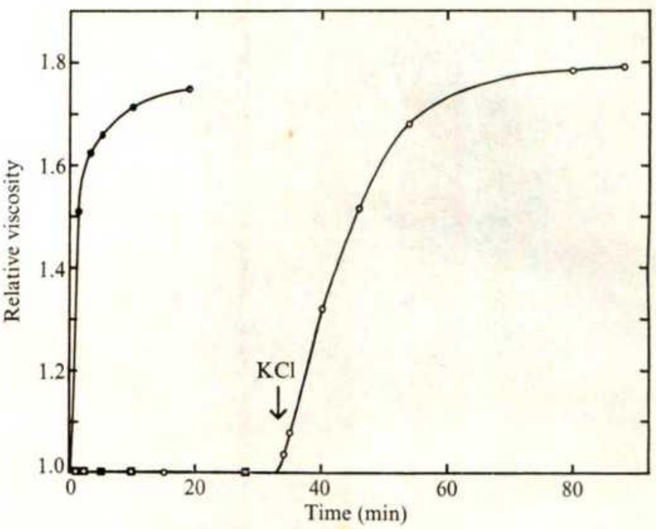

Fig. 1 Increase in relative viscosity of an actin–spectrin mixture. The viscosities of protein solutions containing actin alone (○), actin and the haemoglobin-containing fraction (■), and spectrin with actin (●), were observed with time. Spectrin in this case was an unchromatographed fresh extract of human erythrocyte ghosts containing 0.4 g l^{-1} spectrin; G-actin from rabbit skeletal muscle was used at 0.6 g l^{-1}. 2-ml aliquots for viscosity in S buffer (see Table 1) were introduced into an Ostwald viscometer maintained at 30±0.1 °C, and the flow time for the sample was measured at intervals. The polymerisation of actin alone was induced by addition of KCl to a concentration of 0.1 M at the point indicated by the arrow.

Evidence of specific interaction was also obtained by electron microscopy. Negatively-stained preparations of either the actin or the spectrin showed only small globular subunits (with spectrin we find that aggregation depends critically on the *p*H of the medium[9]); but in the mixture there were abundant filaments (Fig. 2). These seemed to have the form of actin filaments but were predominantly associated in parallel bundles. The polymers could be collected by centrifugation (100,000*g* for 1 h) and gave a clear pellet in conditions in which neither actin nor spectrin alone gave an appreciable residue (Table 1). Analysis of this pellet on SDS gels showed that it contained a preponderance of actin but some spectrin remained even after repeated washing.

Table 1 Formation of sedimentable material in actin–spectrin mixture

Protein	Buffer	Sedimented protein (μg)
G-actin (250 μg)	D	37
G-actin (250 μg)	D+0.1 M NaCl	225
G-actin (250 μg)	S	25
Spectrin (1,040 μg)	S	50
Actin (250 μg)+spectrin (1,040 μg)	S	213
Actin (250 μg)+spectrin (400 μg)	S	63

Solutions were made up in a total volume of 0.5 ml at 4 °C and then warmed to 25 °C for 10 min. They were then cooled to 4 °C and centrifuged at 100,000*g* for 1 h. The sediments were carefully rinsed and then suspended in 0.5 ml of 1% sodium dodecyl sulphate. Protein values were estimated by the Lowry method using BSA as a standard. D buffer; 2 mM Tris-HCl, 0.2 mM $CaCl_2$, 0.2 mM dithiothreitol, 0.2 mM ATP (*p*H 7.4). S buffer; 10 mM sodium phosphate, 0.2 mM dithiothreitol, 0.1 mM $CaCl_2$, 0.2 mM ATP (*p*H 8.2).

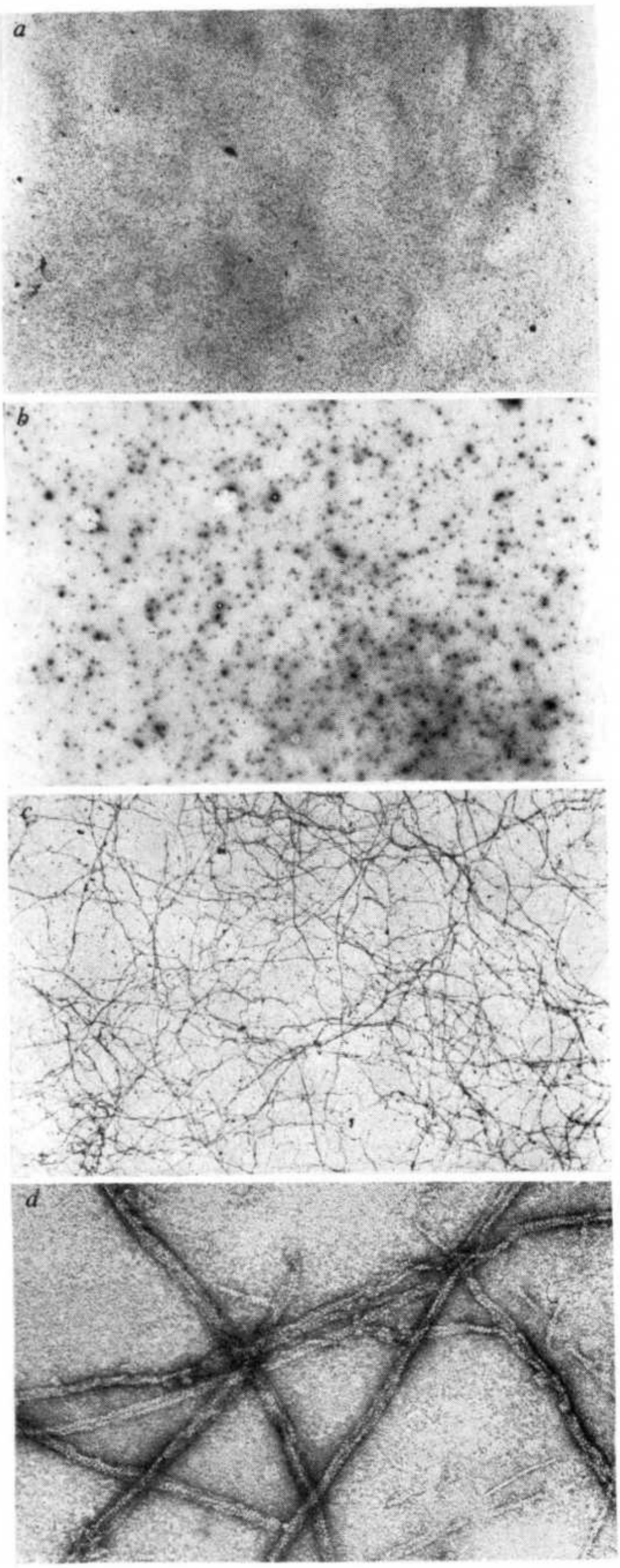

Fig. 2 Generation of filaments in an actin–spectrin mixture. Protein solutions were in S buffer and at similar concentrations to those described in Table 1. Samples were diluted to 0.1 g l^{-1} protein, applied to carbon-coated grids, stained with uranyl acetate and examined in a Phillips 200 electron microscope. *a*, Actin alone; *b*, spectrin alone; *c* and *d*, actin–spectrin mixture. Magnification: *a–c*, ×8,250; *d*, ×90,000.

It therefore seems that spectrin can promote the polymerisation of muscle actin, as judged by the elution profile of the actin in gel filtration, the rapid increase in the viscosity of mixtures, the transformation of the actin into a rapidly sedimenting form, and the appearance of filaments in the electron microscope. This effect could not be reproduced with fractions from the gel filtration column that did not contain spectrin, with haemoglobin or serum albumin in place of spectrin, or with the solution remaining after heating the spectrin to 100 °C.

We believe it is significant that we have been able to observe the interaction between spectrin and G-actin only when the spectrin preparation is fresh, and when it has been extracted from freshly drawn and not stored (for example outdated blood-bank) blood.

After completion of the experiments described above, a report by Tilney and Detmers[10] appeared, which also gave evidence of an interaction between spectrin and actin. In contrast to our results at low ionic strength, these authors observed a diminution in the maximum value of the viscosity generated by the salt-induced polymerisation of G-actin when spectrin was present. These data may not be incompatible with our findings, in consequence of the different ionic strength, the lower spectrin concentration and the possibly different age of the preparations.

These differences dictate caution in extending the results of any experiments of this kind to the conditions existing in the cell. As in other systems involving fibrous proteins[11], spectrin and actin probably give rise to polymorphic aggregates, the nature of which is finely governed by the milieu. It is clear, however, that they do interact and that this interaction may be of great importance, particularly since proteins similar to spectrin are not restricted to the mammalian erythrocyte, and have been found in invertebrate sperm[12], macrophages[13], and sea urchins[14].

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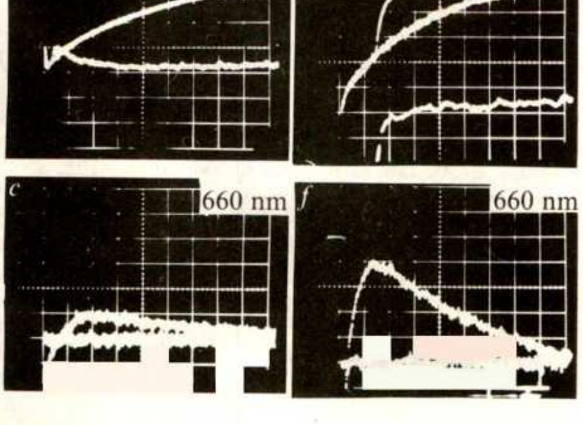
Arrhenius parameters of phototransients in *Halobacterium halobium* in physiological conditions

Halobacterium halobium is indigenous to warm saline pools exposed to bright sunlight. Strains of this bacterium synthesise a purple pigment in the cell membrane (the 'purple membrane'). A single protein, bacteriorhodopsin, is responsible for this characteristic colour, possessing a broad band in the visible spectrum with a maximum at 570 nm (refs 1–3). Pigmented cells utilise solar radiation absorbed by bacteriorhodopsin to create a transmembrane proton gradient which serves as an energy source for ATP synthesis[4,5]. Most information about the mechanism of operation of this light-driven proton pump has been obtained from spectrophotometric studies of suspensions of purple membrane fragments[6–10]. We report here a continuation of studies initiated in this laboratory using modulation excitation[11] and conventional flash photometry[12,13], in which we use the latter technique to observe transient spectral changes in aqueous suspensions of intact cells at physiological temperatures and salinity (basal salt solution).

The principal difficulty in spectroscopic studies of cell suspensions is the intense scattering that obtains in this system. This is particularly acute in flash photometry since long light paths are used generally to monitor the absorption of transients. We have overcome this difficulty in two ways. First, operating our analysing lamp at maximum intensity and using maximum amplification in our recording electronics[13] we could work with relatively short path lengths (5 mm). Second, we reduced scattering by the presence of 40% (w/v) sucrose in the basal salt medium. By this means the refractive index of the medium is increased to approximately that of the cell and scattering becomes negligible. Sucrose was chosen for this purpose since carbohydrates are not metabilised by *H. halobium*[14] and therefore would not be expected to penetrate the cell membrane. We found also that the presence of this concentration of sucrose does not alter the spectrum of the phototransients and in addition the coupled formation of *p*H gradient and synthesis of ATP under continuous illumination proceeds normally.

Fig. 1 Representative appearance and decay profiles of transients in flash-photolysed *H. halobium* cells in basal salt. The photolysing flash was filtered through a Corning 3–69 cutoff filter ($\lambda > 520$ nm). Percentage transmission increases on going from top to bottom. *a–c*, 5 °C; *d–f*, 30 °C. *a*, 410-nm transient: appearance, 0.5 ms per division; decay, 20 ms per division. *b*, 570-nm transient: appearance, 0.5 ms per division; decay, 20 ms per division. *c*, 660-nm transient: appearance and decay, 20 ms per division. *d*, 410-nm transient: appearance, 0.1 ms per division; decay, 5 ms per division. *e*, 570-nm transient: decay at 0.1 and 5 ms per division (due to light scattered from the flash reaching the photomultiplier (uppermost trace) grow-in at this wavelength could not be resolved). *f*, 660-nm appearance and decay, 5 ms per division.



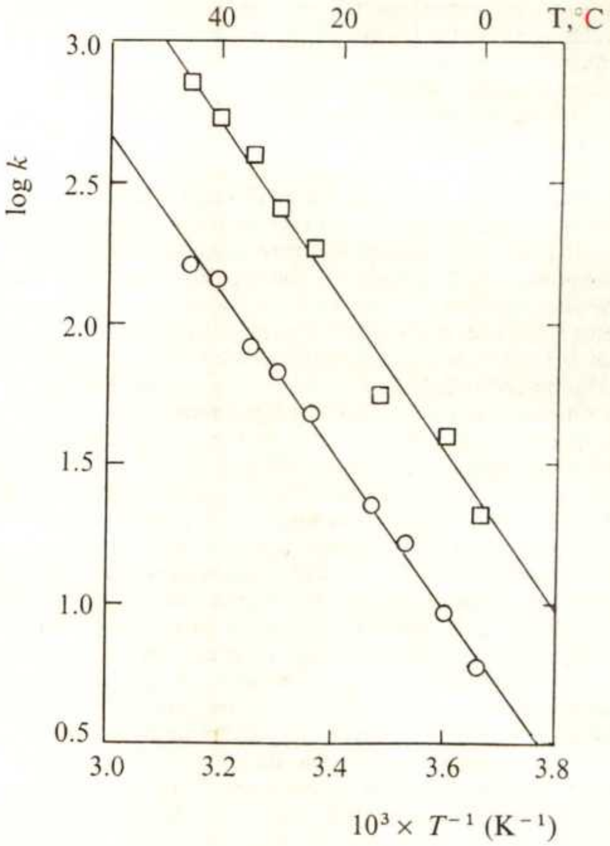


Fig. 2 Arrhenius plot of first-order rate constants for decay of 410-nm phototransients in whole cells (○) and purple membrane fragments (□). Cells were suspended in basal salt, purple membrane fragments were suspended in water.

Transient spectra recorded from flash-photolysed cells in basal salt were essentially identical to those recorded for aqueous suspensions of purple membrane fragments[12,13], exhibiting two chromophores with maxima at 410 and 660 nm respectively, and concomitant depletion centred at the absorption maximum of the initial state chromophore at 570 nm. An isosbestic point was found at 458 nm. Neither of the transient chromophores represent primary photochemical species since relatively long grow-in times were observed (Fig. 1). A similar long grow-in of the 570-nm depletion is also apparent. This is a characteristic of purple membrane suspensions, and evidence was obtained that this phenomenon is a consequence of an overlap from a shorter lived phototransient (maximum about 530 nm) which is the precursor of the 410-nm species[12,13].

Cell suspensions were studied in the temperature range 0–45 °C, about 37 °C being optimum for cell growth. At each temperature decay of the phototransients and the concomitant reappearance of the ground state chromophore obeyed first order kinetics with equal half lives. The maximum intensities of the 410 and 570-nm transients were relatively insensitive to temperature, while that for the 660-nm transient decreased with decreasing temperature, becoming undetectable at 0 °C. Grow-in and decay times of all three phototransients increased with decreasing temperature. The first-order rate constants for their decay conform to an Arrhenius plot (Fig. 2) with energy of activation $E_a = 13$ kcalorie mol^{-1} and entropy of activation $S_a = -7$ e.u. Results obtained with purple membrane suspensions in water are also presented in Fig. 2, and yield similar activation parameters ($E_a = 13$ kcalorie mol^{-1}, $S_a = -4$ e.u.). At the same temperatures phototransients in intact cells exhibited longer half lives than those in purple membrane suspensions, possibly a consequence of the difference in suspension medium (Fig. 2 legend).

As found for purple membrane suspensions[13], flash photometry using polarising filters showed all three phototransients in intact cells to be polarised. Only small decreases in dichroic ratios were observed during the transient lifetimes showing the bacteriorhodopsin molecule to be essentially immobilised within its membrane.

The transients we have observed clearly relate to the final step of the proton-pumping cycle, although the question as to whether they occupy a common or parallel pathway is under discussion (W.V.S., R. Korenstein and S.R.C., manuscript in preparation). Based on recent Raman studies[15,16], a convincing argument has been presented which identifies the 410-nm and 570-nm chromophores as representing, respectively, free and protonated Schiff bases[16]. The coupled decay of the 410-nm transient and regeneration of the 570-nm chromophore represents a last step in the cycle. This step involves no breaking of bonds or molecular rearrangement and may be expected to be diffusion-controlled, that is dependent on the rate at which protons can diffuse through the membrane (or through the peripheral regions of the protein molecule itself) to the site of the chromophore. The small entropy of activation that we obtain agrees with that which may be estimated from the data of Lanyi for Brownian movement within membrane vesicles from the related *H. cutirubrum* (about -9 e.u.)[17] and is therefore consistent with this proton diffusion model. The activation energy that we obtain is higher than Lanyi's (3–4 kcalorie mol^{-1}) and may indicate that lateral proton diffusion in *H. halobium* is associated with a particle which has a larger effective size than that for rotational diffusion of the perylene molecule used as a probe in the *H. cutirubrum* study. (Alternatively, diffusion within the protein may be the limiting factor.)

Several similarities between bacteriorhodopsin and vertebrate rhodopsin have been identified[1,4,7]. Our results, however, point to two significant differences. First, whereas rapid rotation of the pigment within its membrane is possible in vertebrate rhodopsin[18,19], bacteriorhodopsin is immobilised within its membrane. The second difference relates to proton uptake. In vertebrate rhodopsin, uptake of one proton per rhodopsin molecule is associated with the thermal relaxation of metarhodopsin I → metarhodopsin II (refs 20 and 21). First-order treatment of the spectral intensities associated with these intermediates yields $E_a = 31$ kcalorie mol^{-1} and $S_a = +55$ e.u. (ref. 22). Analysis of the data on proton uptake[21] yields consistent values of $E_a = 27$ kcalorie mol^{-1} and $S_a = +42$ e.u. The magnitude of these activation parameters for proton uptake in vertebrate rhodopsin therefore shows significant molecular rearrangement in the rate determining step (release of phospholipids has also been identified at this stage[22]). In contrast, proton uptake in bacteriorhodopsin is quite different involving, as we have shown, small activation parameters and therefore little molecular rearrangement in its rate determining step.

Measurement of *p*H and ATP synthesis in cell suspension under continuous illumination was carried out by Arlette Danon. W.V.S. was on sabbatical leave from the Department of Physical Sciences, Chicago State University. This research was supported by a grant from the United States–Israel Binational Science Foundation and the West German Ministry of Science and Technology.

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Nanosecond flash photolysis of rhodopsin

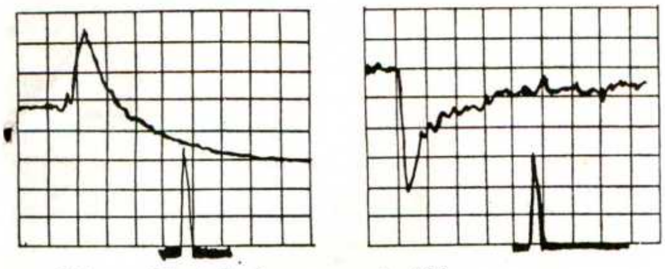
THIS note presents results from a neodymium laser flash spectroscopy study of bovine rod outer segments (ROS), the only chromophore of which is rhodopsin. Previously unreported transients are interpreted in terms of a charge-transfer excited state which gives rise to radicals and/or triplets.

The change in the absorption spectra of visual pigments such as rhodopsin after irradiation is a much studied process following the original work of Wald[1]. These studies have used predominantly conventional spectrophotometry, or conventional flash photolysis with micro- or millisecond resolution times. To study the processes occurring after the pigment absorbs a photon (rhodopsin → prelumirhodopsin (PLR) → lumirhodopsin (LR)) low temperatures were used.

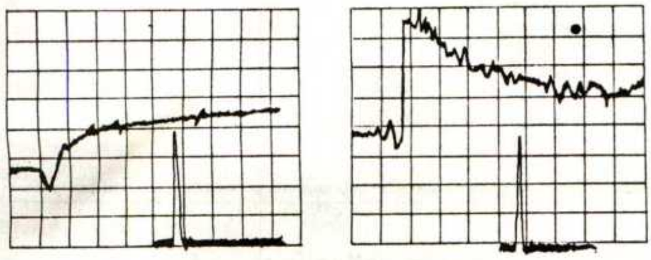
Recent reports of work at room temperature or above using nanosecond resolution times or less were limited to a narrow range of wavelength detection. Cone[2] reported transient absorption changes at 510 nm and 580 nm, and concluded that PLR decays to LR with a half life of 170±30 ns and LR decays to metarhodopsin I (MRI) with a half life of 50±20 µs. Busch *et al.*[3] studied rhodopsin with a mode-locked laser and monitored transient absorption changes at 560 nm. They estimated that PLR is formed in 6 ps or less and decays with a lifetime of about 30 ns. Rosenfeld *et al.*[4], using an N_2 laser to excite rhodopsin in the β band (330 nm), were able to measure transient absorption changes at wavelengths between 575 and 650 nm. They excited outside the main α absorption band of rhodopsin and consequently required the use of high optical densities of rhodopsin at wavelengths corresponding to the α band (4.0 at 498 nm). They estimated a PLR lifetime of ~ 50 ns and a maximum near 580 nm.

We used a Nd laser flash apparatus[5]. Most of our results concern the use of the 530 nm excitation line obtained by frequency doubling the laser output. A few results using 265-nm and 353-nm excitations are also included. Spectral measurements were made over the wavelength range 320–610 nm. The ROS (ref. 6) were solubilised with 3% Emulphogen in Tris buffer at *p*H 8.2. For the various samples of ROS used the ratio A_{278}/A_{500} was in the range 2.0–2.2.

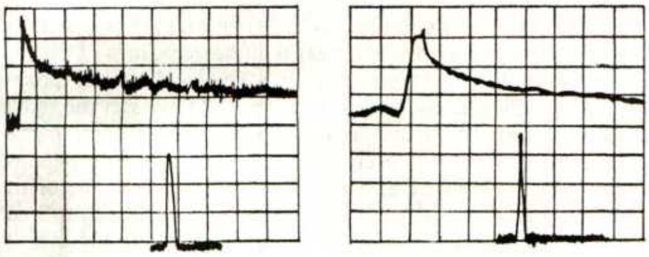
Our observations are best considered in terms of typical oscillograms at varying monitoring wavelengths. Fig. 1 shows such oscillograms at 570 nm, 500 nm, 450 nm and 350 nm. At 570 nm we observed (Fig. 1*a*) an immediate increase in absorption. This transient absorption decays with a half life of 125±25 ns giving a final increase in transmission compared with the original absorbance. Laser excitation at 353 nm and at 265 nm leads to oscillograms similar to that at 570 nm. Monitoring at 500 nm, with 530-nm excitation (Fig. 1*b*), we observed a rapid decrease followed by an increase in absorption with a half life of ~ 125 ns. At 450 nm (Fig. 1*c*), an initial fast increase in absorption is followed by a further increase in absorption with a half life of ~ 125 ns. We estimate the lifetime of this secondary transient to be between five and several tens of µs. At 350 nm (Fig. 1*d* and *e*) a short lived species with half life of ~ 125 ns, is followed by a longer lived transient with a half life of ~ 5 µs. In order to test for the



a 570 nm First flash *b* 500 nm



c 450 nm *d* 350 nm



e 350 nm *f* 570 nm Second flash

Fig. 1 Oscilloscope traces of absorption changes following 530-nm laser excitation of ROS in 3% Emulphogen. Horizontal displacements; time (1 large division equals 100 ns except for *e* where it equals 500 ns). Displacements vertically upward; decreasing transmission (1 large division equals 5.88, 1.96, 6.55, 1.05, 1.08 and 5.88% change in transmission for *a*–*f* respectively). A fresh solution was used in each case except for *f*. The ROS concentration for *a* and *f* was ten times that for *b*–*e*. The traces at the bottom of each photo arise from the photodiode which monitors the laser flash intensity from flash to flash. The initial increase in transmission in *b* and *c*, over within 50 ns, is due to scattered laser light.

possibility of two photon effects, the laser intensity was varied over a sixfold range. Since only linear variations in absorbance with light intensity were obtained, two photon effects are not occurring.

Figure 2*a* shows the difference spectra obtained 50 and 800 ns after the beginning of the flash. We have assumed that the transient absorption band at 550 nm is symmetrical and have used the fact that the transient spectrum after 50 ns is isosbestic with rhodopsin at 537 and 479 nm. Even if our assumption is incorrect, the position of the various maxima we observe is unaffected, only the relative intensity of the bands is changed. Figure 2*b* shows the transient spectra after these corrections. Table 1 shows our estimated extinction coefficients at these maxima using a value of 41,000 M^{-1} cm^{-1} as the extinction coefficient of rhodopsin[7].

After the ROS have been illuminated, similar transients to those above are observed over the wavelength range 320–400 nm; Fig. 1*f* shows a typical oscillogram at 570 nm. The unbleached ROS show an initial increase in absorption (Fig. 1*a*). Bleached ROS, that is, after 1 or more flashes or after standing in normal room lighting for several minutes, show an initial increase in absorption but no subsequent decrease compared with the original absorption (Fig. 1*f*). The transients we observe from bleached ROS may arise from pararhodopsin[8].

The usual structural interpretation of the spectral changes after the initial absorption of light by visual pigments such as rhodopsin, is that following the initial 11-*cis* to *trans* isomerisation of retinal (to form PLR), there are various conformational changes of the protein, opsin, the consecutive transients formed being called LR, MRI and metarhodopsin II, and so on.

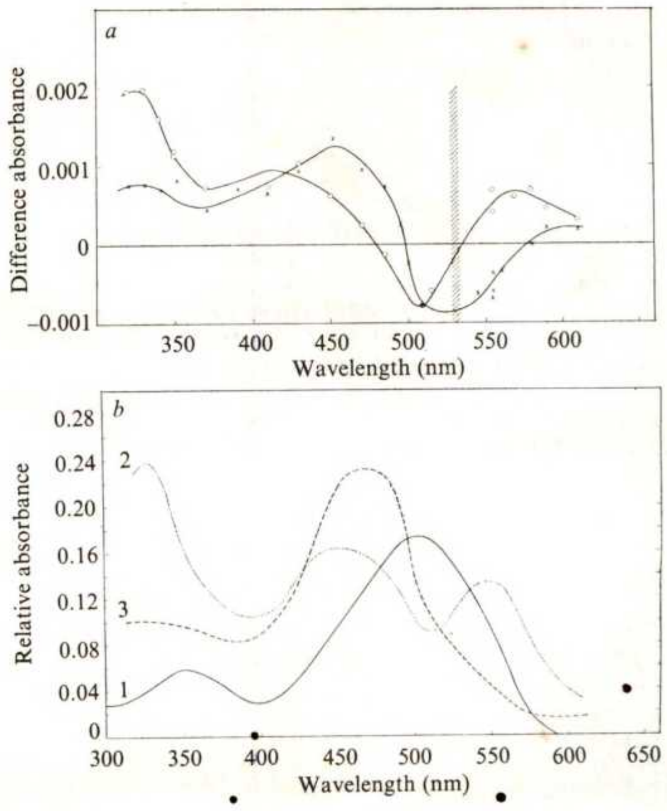
The earliest transient from ROS (curve 2 in Fig. 2*b*) has a maximum absorption on the long wavelength side of the rhodopsin absorption, but also shows two additional bands at about 450 and 330 nm. A long wavelength (550 nm) absorption is well known at low temperatures[1] and was called PLR. The second formed transient spectrum (curve 3 in Figure 2*b*) shows a single maximum absorption on the short wavelength side of rhodopsin and seems to correspond to the LR (or MRI) absorption at low temperatures[1]. Thus, while some of our results might seem to be in agreement with the previous low temperature studies in terms of PLR and LR (or MRI) the extra peaks observed immediately after the laser flash do not seem to fit in with the accepted scheme. Yoshizawa[9,10] has observed an intermediate absorbing at ~ 430 nm (hypsorhodopsin) on irradiation of rhodopsin at liquid helium temperature. This is converted into PLR on raising the temperature or on absorption of light of wavelength below 430 nm. We do not believe that the peak we observe at 450±10 nm is the same species since it is formed and decays at the same rate as the peaks at 550 and 330 nm.

Table 1 Maximum molar extinction coefficients at 293 K

Species	Wavelength (nm)	Extinction coefficient (M^{-1} cm^{-1})
Rhodopsin	500	41,000*
First transient	550±10	31,500±3,000
	450±10	38,800±4,000
	330±10	56,100±5,500
Second transient	470±10	54,900±5,500

*See ref. 7.

Fig. 2 *a*, Absorption changes following 530-nm laser excitation of ROS in 3% Emulphogen. ○, ~50 ns and ×, 800 ns after the beginning of the laser flash. *b*, Corrected absorption spectra of first (2) and second (3) transients. The absorption spectrum of the original ROS is also shown (1).



We believe that tryptophan is involved with retinal in the chromophore. The results of Lewis and Spoonhower[11] using resonance Raman spectroscopy imply the existence of a strong complex between retinal and a tryptophan residue in rhodopsin. Shirane[12] claims from electron spin resonance evidence that the excitation energy absorbed in the range 450–600 nm by frog rhodopsin results in the formation of the triplet state of tryptophan. Various transient absorption spectra from tryptophan in aqueous solution have been reported by Santus and Grossweiner[13]. A short lived transient absorption with a maximum at 460 nm was assigned to the triplet state of tryptophan. We have previously[14] reported a transient absorption of 11-*cis* retinal at 445 nm which we assign to the retinal triplet state. The first transient absorption we now observe on flash photolysis of ROS includes a maximum at 450±10 nm. Santus and Grossweiner[13] assign a transient absorption at 340 nm and a weaker transient at about 600 nm with a shoulder at 550 nm to the radical cation of L-tryptophan and 1-methyl-L-tryptophan. The wavelengths of the maxima of these two transient absorptions are close to the maxima at 330±10 and 550±10 nm we observe as part of the first transient obtained on flash photolysis of ROS. The second transient (Fig. 2*b*) shows only one maximum, at 470±10 nm. Santus and Grossweiner[13] reported an absorption at 485 nm after flash irradiation of L-tryptophan at *p*H 5.4 at 25 °C which they assign to the neutral radical formed by deprotonation of the radical cation of tryptophan.

Charge transfer involving an electron donor–acceptor situation in which the complexes are stable in the ground state are well known. They give transient spectra, showing features of both radical cations and anions and of excited states of the individual components of the complex[15]. Excitation at any wavelength where the charge transfer complex absorbs may lead to a single species and the lifetime of the individual bands associated with this species would be identical. Furthermore, Ishigami *et al.*[16] and Mendelsohn[17] have both suggested that a charge transfer complex between indole rings and retinal exists and is reponsible for the major absorption band in the region 500–600 nm for such complexes.

We suggest that an excited state of the charge transfer complex rhodopsin may be the primary intermediate involved in the vision process. The products of this excited state may be radicals and/or triplets rather than only conformational changes of opsin. The primary intermediate (first transient) bears the characteristic absorption bands of tryptophan, retinal radical ions and triplet states. Since excited states are short lived even at very low temperatures, it is not surprising that the first transient we observe does not appear in the low temperature spectrum reported by previous workers after low intensity irradiation of rhodopsin.

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Equatorial X-ray reflections and cross arm movement in skeletal muscle

WHEN resting skeletal muscle is excited or goes into rigor, there is a characteristic change in the ratio of the intensities of the two principal equatorial reflections, I_{11}/I_{10}. This has been interpreted simply as reflecting movement or transfer of mass in the radial direction from the thick to thin filaments[1,2]. Other, higher orders, have, however, been observed[1,3], and a more detailed interpretation is possible, which should eliminate some of the ambiguity that results from using only two reflections. Since there is considerable evidence that the myosin filament is actually three or four stranded[4–6], rather than two stranded as originally proposed, reinterpretation is necessary. Further, studies of X-ray diagrams from muscle in the presence of ATP analogues suggest that at least in some cases, equatorial intensity ratios intermediate between rigor and relaxed do not arise from a mixture of those states, but rather from a state with different structural properties[7–9].

The number of observed equatorial reflections is very limited (compare Table 1). This, plus the difficulty of determining the phases, means that a completely unambiguous answer is not possible; however, various models of the packing of the actin and myosin within the hexagonal filament lattice, can be described and the mass projected on to a unit cell in the equatorial plane. From such models, predicted phases and relative intensities can be calculated and then compared with experimental results to eliminate some possibilities. Five such models are shown in Fig. 1 and the predicted intensities are given in Table 1, together with experimentally measured intensities from a frog sartorius muscle (*Rana pipiens*) at a sarcomere length of 2.2 μm.

The difficulty of deciding between different models solely on the basis of I_{11}/I_{10} can be seen by comparing the last four columns in Table 1. The four models all give reasonable 'relaxed' ratios. The higher order reflections provide more information and allow certain distinctions to be made. Models II and III are unsatisfactory in predicting appreciable intensities for the higher orders. Any model with short cross arms will probably have a fall off in intensity much sharper than observed experimentally. Models IV and V, with longer arms, are better in predicting higher orders, though refinement of the models would clearly be necessary for a precise fit. It would, however, be easier to fit the experimental equatorial data by using models with cross arms projecting to a radius of 180–200 Å. Most importantly, such models with longer arms will fit axial diffraction data better. Cross arm packing similar to either model IV or V will fit experimental intensity measurements of the first-layer line from frog muscle, assuming that the thick filaments are either three or four stranded (R. W. L. and G. H. Cohen, in preparation). Thus, both the axial and equatorial

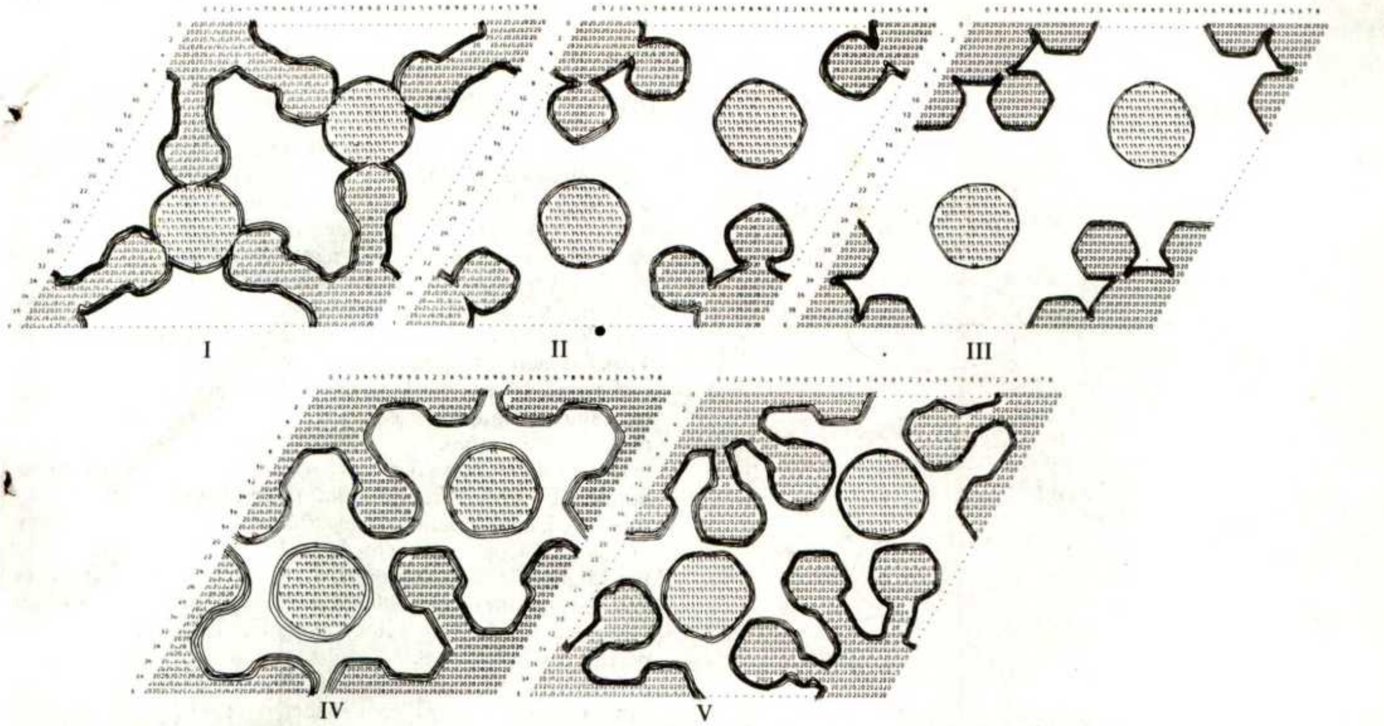

Fig. 1 Five models of the packing of myosin cross arms within the unit cell of the hexagonal lattice of filaments, projected on to the equatorial plane (equivalent to looking along the fibre axis). The backbone of the thick filament is located at the corners of the unit cell. The projection of the two actin filaments per unit cell are the circles at the trigonal points. The actin filament is assumed to project to a circle of radius 40 Å, whereas the core of the myosin filament is 60 Å radius. The myosin cross arms assume that the two subunits lie side by side, to fuse to one, almost circular, head. The dimensions, and relative mass within a myosin 'molecule' approximate reported values[10]. The actin–myosin mass ratio has a value of 0.25, which is close to what one expects in the region of overlap of a frog sartorius muscle with a sarcomere length of 2.2 μm. In models II and III, the globular cross arms of myosin project to a radius of 130 Å, whereas in models I, IV, and V the arms project to a radius of 180 Å. The side of the unit cell is 400 Å. Model V is a projection expected from a three-stranded thick filament, with one of the cross arms in the *10* plane (at an azimuthal angle of 0°). Models III and IV have cross arms at an azimuthal angle of 0°, while I and II form azimuthal angles of 30°. Model I gives an I_{11}/I_{10} that is typical of rigor muscle, while models II, III, IV, and V all give ratios typical of resting muscle.

diffraction patterns can be fitted assuming that the cross arms project to a radius of the order of 180–200 Å. In insect flight muscle it also seems that models in which the cross arms extend far from the thick filament[12] can fit the relaxed pattern.

Thus the difference between the I_{11}/I_{10} from models IV and I is most provocative. The only difference between the two models is that in model IV the myosin cross arm forms an azimuthal angle of 0° with the *10* plane, while in model I, the angle is 30° (that is, the cross arm is in the *11* plane). This allows for the strong possibility that the change in I_{11}/I_{10} when a muscle is excited, arises mainly from azimuthal variations in the configuration within the equatorial projection, with little radial movement outwards from the thick filament (shown schematically in Fig. 2). This suggests that excitation of the muscle causes azimuthal disordering of the myosin cross arm with respect to the helical packing of the thick filament backbone, with simultaneous weakening of the relaxed layer-line pattern. Those cross arms which attach to actin can be further twisted, azimuthally and axially (that is, in a plane perpendicular to the page), to produce the slewed actin–myosin rigor state reported by Moore *et al.*[13]. This would explain many of the changes seen in X-ray diagrams on excitation, and generate

Table 1 Relative equatorial intensities*

Model	I	II	III	IV	V	
Azimuthal angle	30°	30°	0°	0°	0°	
Radius	long	short	short	long	long	
Rotational symmetry	6	6	6	6	9	
						Experimental
Reflection						
10	100(0)	100(0)	100(0)	100(0)	100(0)	100
11	324(0)	47(0)	33(0)	30(0)	25(0)	44
20	46(π)	7(π)	3(π)	36(0)	5(0)	9
12, 21	61(0)	3(π)	10(π)	13(π)	36(0)	9
30	15(π)	0(0)	18(0)	150(0)	35(0)	12
22	33(0)	2(0)	1(π)	11(0)	19(1.4)	20
13, 31	30(π)	0(0)	0(0)	21(π)	84(2.1)	

* Intensities are calculated from the amplitudes, F, assuming that the experimentally observed pattern is equivalent to a rotation of the unit cell around the fibre axis. Thus $I_{hk} \propto (F_{hk})^2/\sqrt{(h^2 + k^2 + hk)}$. The predicted phases, in rad, are given in parentheses. They are very model dependent for higher orders.

Amplitudes and phases were calculated from the models in Fig. 1 using the fast Fourier program CHAFF described by Bantz and Zwick[11]. The *22*, *13*, *31* reflections could not be resolved experimentally.

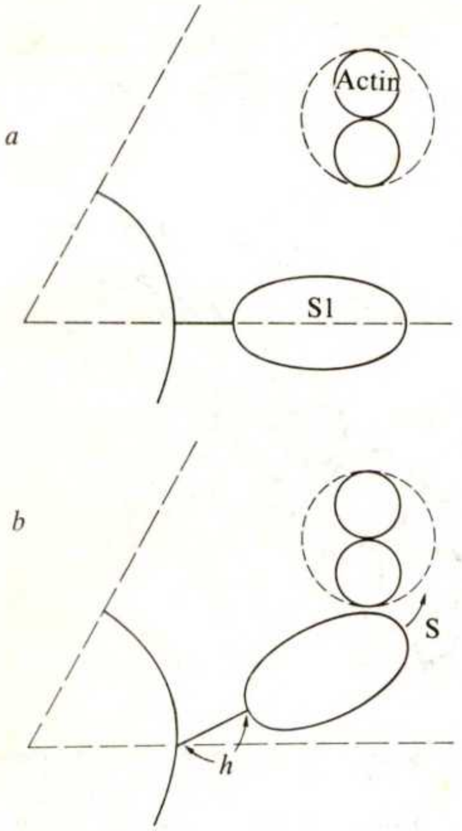

Fig. 2 Schematic representation in the equatorial plane of possible two-dimensional organisations of actin and myosin molecules. *a*, A section of the unit cell in Fig. 1. A single subfragment 1 (S1) moiety, containing one actin-binding site and one ATPase site, projects from the thick filament backbone to a radius of 210 Å, centred in the *10* plane for demonstration. Two actin monomers are shown at the trigonal point, in an orientation favouring creation of an actin–myosin link. *b*, When the muscle is excited, the S1 moiety can be displaced azimuthally from its rest position. (Note that S1 in the 'rest' state is actually fluctuating about the single rest state shown in *a*. The azimuthal displacement may simply represent its immobilisation by binding to actin.) This causes a change in I_{11}/I_{10}. On binding to actin, further movement may occur (arrow (S)), as the moiety slews round the actin within the equatorial plane, as well as in the axial direction (perpendicular to the page). Flexibility is allowed in the entire subfragment 2 region, between the two arrows, *h*.

tension in a way that would easily fit into many physiological schemes.

Drs L. Yu and R. N. St Onge supplied the spectra used to obtain the experimental numbers in Table 1. This work, using an electronic position sensitive detector, will appear elsewhere.

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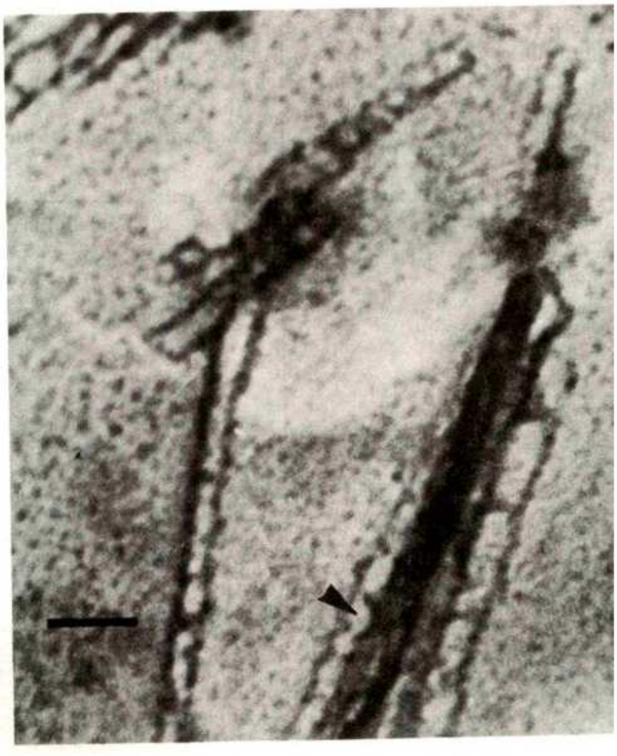
Structural subunit in prisms of immature rat enamel

THE prisms of rat enamel, like those of other mammal are composed of lath-like crystals with maximum width of about 30 nm, arranged longitudinally in an organi matrix. The matrix is considered either to contain group of fibrils or septae which orientate the crystallites, or t consist of an amorphous gel. The first view is based on th fact that pairs of electron-dense linear structures have bee seen in fixed demineralised sections of immature ename These structures were about 5 nm wide and 15 nm apar and parallel with the long axis of the prism. Furthermor irregularly-arranged cross bridges have been described ru ning between them[1,2,3]. Physical techniques of structu determination, however, have failed to show evidence periodicity in demineralised immature enamel[4] and this h supported an alternative view that the matrix is an amo phous thixotropic gel[5]. This latter concept is consistent wit the change in matrix composition when enamel mature and a simultaneous reduction in volume[7]. It does no however, offer a satisfactory explanation of the orientati of the crystallites.

I have found another inconsistency—isolated prisms fro immature enamel, which had been suspended in saturat calcium phosphate solution, could withstand ultrasonic tion for up to 1 h. This could not be attributed to an inte lacing of the crystallites, because if 8 M urea soluti

Fig. 1 Electron micrograph of demineralised crystallites isolated from prisms of immature rat enamel. Bar represents 100 nm. The arrow shows the junctional region between adjacent helices. The crystallites were obtained from albino rats (150 g). A 2-mm section of the soft enamel adjacent to the mature enamel was ultrasonicated for 10 min in 0.5 ml of distilled water which had been saturated with calcium phosphate. The crystallites were washed once with 1.5 ml of the suspending solution and deposited on copper grids with Formavar films. After drying, the grids were placed in a solution of 4% glutaraldehyde–0.1 M EDTA (pH 7.4) and withdrawn at 10-s intervals after 1 min. The grids were examined at ×7,500 with an AEI EM6 B electron microscope to find suitable areas for photography.



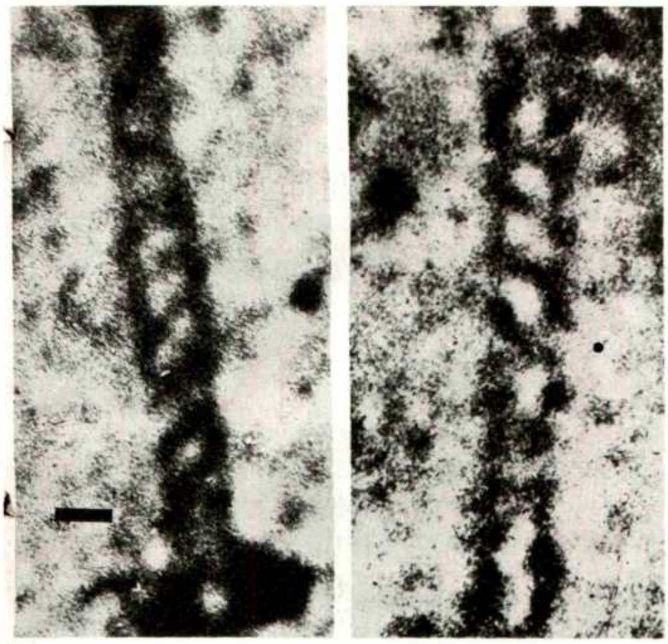


Fig. 2 Two selected areas of demineralised enamel crystallites showing helical arrangement. The bar represents 30 nm. Magnification in the electron microscope was ×7,500 and subsequent enlargement was photographic to minimise beam damage to the unsupported specimens.

saturated with calcium phosphate was used to treat prisms either before or after ultrasonication they rapidly fell apart to yield individual crystallites. Urea (8 M) causes hydrogen bonds to break and this may have produced the effect on the prisms, either by allowing a structural network to separate, or by rendering a cementing substance more soluble. Sodium chloride solutions have been used widely to precipitate the soluble components of enamel matrix[8], but a range of concentrations (0.3–2.0 M) failed to inhibit the action of 8 M urea solution, so it was concluded that a cement substance was not being made more soluble, and therefore that a structural framework was probably involved, part of the matrix being in that form.

To investigate this possibility further, isolated prisms which had partially unwound into separated crystallites were dried on to Formavar films on electron microscope grids, and demineralised carefully. As Fig. 1 shows, double lines of electron-dense material with cross bridges were observed, similar to those described in sectioned material. After examining many electron micrographs I concluded that this material consisted of double helices which seemed to have been wound around the crystallites. Selected areas which confirm this view are shown in Fig. 2. The helices varied in overall width from 15 to 30 nm and their pitch varied from 30 to 90 nm. The thickness of the coils of the helices was about 5 nm. Some appearances in Figs 1 and 3 suggested that bonding had existed where turns of helices on adjacent crystals came into contact. The variable pitch of helices would mean that they would not show periodicity and therefore would not be detected by physical techniques previously mentioned.

These observations and this interpretation could provide the basis for an explanation of the structural framework postulated here. This framework would consist of groups of helices whose long axes would be approximately parallel to one another and to the long axis of the prism. There would be multiple bonds betwen adjacent helices where their coils came in contact. Crystals threaded through the helices would brace the structure and give compressive strength. Against this view it could be argued that the structures described here were not present *in vivo*, but were aggregates of amorphous material which had been released during preparation, and adsorbed on to the crystallites before dissolution. It is unlikely, however, that random forces could produce such consistent appearances. Furthermore, when the suspensions of rat enamel were made in 1.2 M phosphate buffer at *p*H 6.8, the helical structures could still be observed after demineralisation. As is well known, strong phosphate buffers desorb organic aggregates from hydroxyapatite[9,10], so that it is unlikely that aggregates could form in such a solution.

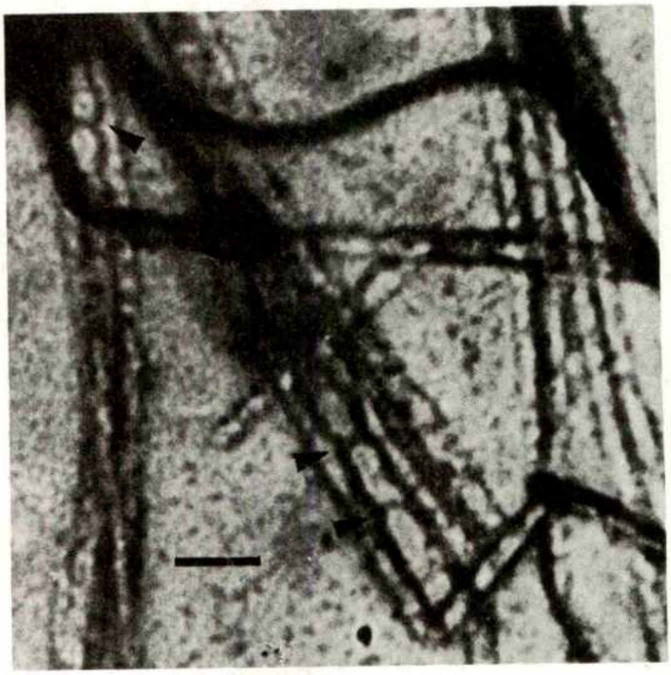


Fig. 3 Demineralised enamel crystallites showing evidence of bonding (arrowed) between helices on adjacent crystallites. The bar represents 100 nm.

It is interesting that structural helices with coils 5 nm thick have been described in F actin[11] (a double helix) and certain bacterial flagella (four or five helices)[12]. The benefits and efficiency inherent in the use of helices as the basis of biological structures have been pointed out by Crane[13] and Kushner[14]. It has also been suggested that some electron micrographs of the microtubules found in various animal and plant cells could be interpreted as showing helical arrangements of subunits, 5 nm in diameter[14]. It is possible therefore that the structures described here are the remnants of microtubules produced by the ameloblasts in the early stages of enamel formulation. Jessen[15] has described groups of extracellular microtubules associated with the forming ends of rat ameloblasts. Furthermore, Jessen[16] has demonstrated the presence of helical structures located in the mitochondria of the ameloblasts. They were of similar magnitude to those described above. The presence of such an unusual structure within the cells responsible for the formation of enamel provides strong support for the idea that the helices seen on enamel crystallites are an integral part of its structure at least at some stage in its development.

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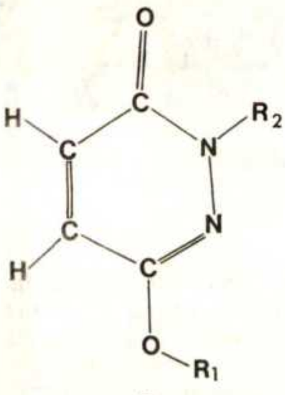
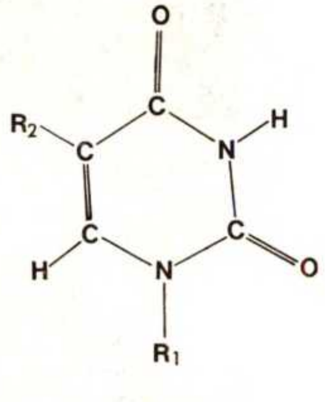
Is maleic hydrazide a pyrimidine or purine analogue?

MALEIC hydrazide (structure I, $R_1=R_2=H$) is well known in agriculture as a growth inhibitor, but its mechanism of action is uncertain. Its close structural resemblance to the pyrimidine base uracil (structure II, $R_1=R_2=H$) has led to the suggestion that it replaces uracil in the RNA molecule[1,2], and although this theory is not universally accepted[3], maleic hydrazide is known to be incorporated into RNA[2]. The crystal structure of maleic hydrazide has now been elucidated (a full report will be published elsewhere) and a model of the molecular structure as found in the crystal has been used in attempts to form base pairs with the nucleic acid bases. The geometrical criteria of hydrogen bond formation suggests that maleic hydrazide can be regarded as either a pyrimidine or a purine analogue



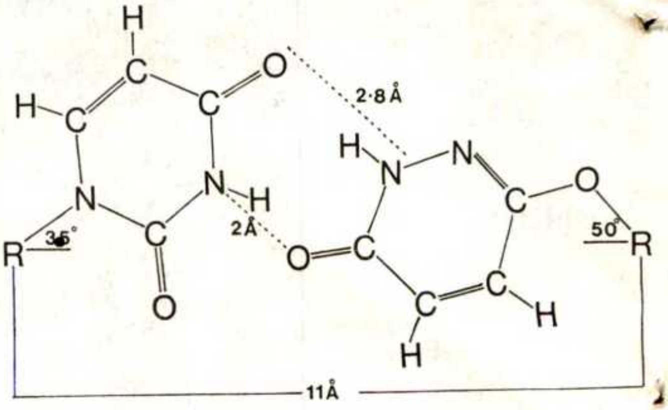
I



II

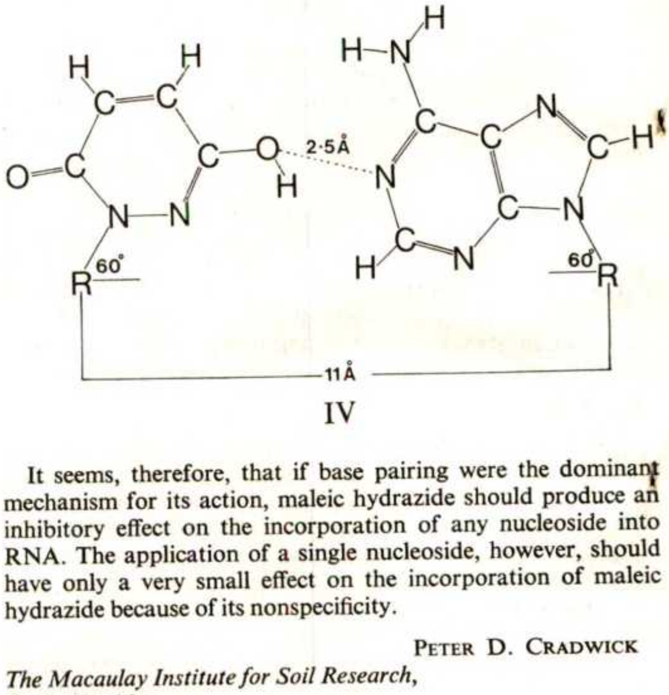
Maleic hydrazide crystallises in the space group $P\bar{1}$: $a=5.832$; $b=5.782$; $c=7.306$ Å; $\alpha=78.95°$; $\beta=99.54°$; $\gamma=107.23°$; $Z=2$. Three dimensional intensity data were recorded on Weissenberg film packs using Cu$K\alpha$ radiation and were measured photometrically. The structure was solved by application of the symbolic addition prodedure[4] and the rotation function[5]. Using three symbols, 81 reflections were phased, requiring the calculation of eight E maps, and one of these was found to be correct on the basis of orientation information provided by the rotation function. This structure was refined by the full matrix least squares procedure to a conventional R factor of 0.063, and corresponds to structure I.

The incorporation of a base or base analogue into the RNA structure requires the formation of a nucleoside, for example, uridine (structure II, R_1=ribose; R_2=H) resulting in a carbon–nitrogen bond between the base and ribose. Maleic hydrazide can form a bond either through its hydroxyl oxygen atom, giving the nucleoside analogue I (R_1=ribose, R_2=H) or through the NH group to form I (R_1=H; R_2=ribose). In the former case, base-pair formation with adenine is possible only if the O–R ··· R angle (shown as 50° in structure III) is increased to 100°. This angle is normally about 50° for Watson–Crick base pairs, and though distortions of about 20° are known[6], an angle of 100° would require major distortion of the ribose–phosphate chain. Base-pair formation is possible, however, with the pyrimidine bases uracil or thymine (structure II: $R_1=H$; $R_2=CH_3$)



III

Nucleoside formation through the nitrogen atom, on the other hand, could result in base-pair formation with adenine as shown on structure IV. Furthermore, with maleic hydrazide in the diketo form, a base pair of similar geometry is possible with guanine.



IV

It seems, therefore, that if base pairing were the dominant mechanism for its action, maleic hydrazide should produce an inhibitory effect on the incorporation of any nucleoside into RNA. The application of a single nucleoside, however, should have only a very small effect on the incorporation of maleic hydrazide because of its nonspecificity.

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Corrigendum

In the article "Human breast cancer responsive to androgens in long term tissue culture" by M. E. Lippman, G. Bolan and K. Huff (*Nature*, **258**, 339; 1975), the authors omitted to thank the Michigan Cancer Foundation and Dr M. Rich for supplying them with a starter culture of their MCF 7 cell line.

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riability of mmals

iability of Mammals. By A. V.
lokov. Translated from Russian.
xv+350. (Amerind : New Delhi.)
50.

s book deals with an increasingly
need for a unified approach to the
tion of data on population mor-
ogy. In the past this has been in
rovince of geneticists, comparative
ists, zoogeographers and taxo-
, but now data are collected
uired by a far wider spectrum
gists. In synthesising this re-
ofessor Yablokov has drawn
on data collected by Russian
sts which are not readily avail-
in the west. Useful as that
ach is, it inevitably means that
data must be accepted at face
for accuracy of measurement
no allowance made for error; the
does not caution the reader to be
is guard to interpret the facts
dingly. Nevertheless, a great deal
rk has yet to be done before a
al theory of variability can be
ced. and one must beware of
ing the baby out with the bath-

first part of the book lays the
ations for a discussion of the
veness of variability by using a
ent of variation of a series of
as an index of the different
ological conditions encountered by
ifferent populations of the same
pecies. Since few distribution curves
given traits correspond to a normal
distribution, the best means for expres-
ing differences is a problem yet to be
ved by morphologists. The dimen-
ionless coefficient (the standard devia-
on divided by the sample mean and
pressed as a percentage) is used
oughout the book to compare widely
erent traits between populations
in a species, within a genus, be-
en closely related genera, between
families and within an order. The
ult of the author's examination of
its in the Pinnipedia indicates that
coefficient of variation between two
ulations may be significantly grea-
than the coefficient of variation for
ulations from two different sub-
ilies. Professor Yablokov argues for
tionalisation of the classification of
ability in various independent ways
that the processes of evolution of
rent populations can be directly
pared. This is particularly valuable
n comparing the variability of
ral and experimentally controlled
lations and that between popula-
s experiencing clearly distinguished
different environmental condi-

A heavy, laboured style makes for difficult reading, but this may be a result of the translation. Several line diagrams have insufficient legends and the halftone plates are so badly reproduced as to be almost without value. Nevertheless, the book is a useful starting point for the serious student of population evolution and mammalian — in particular pinnipede — biology, and it should fulfil a role in describing the baseline of this important area of biology.

D. Michael Stoddart

Johannes de Ketham's *Fasciculus medicine* shows the first printed illustration of a human dissection, attributed by some scholars to the artist Gentile Bellini. Originally published in 1491 in Venice shortly before Copernicus became a medical student in nearby Padua. (Yale Historical Medical Library.) *The Nature of Scientific Discovery*. (A symposium commemorating the 500th anniversary of the birth of Nicholas Copernicus.) Edited by Owen Gingerich. Pp. 616. (Smithsonian Institution Press : Washington, DC, June 1975.) $15.00.

Energetic perspective

Perspectives on Energy: Issues, Ideas and Environmental Dilemmas. By Lon C. Reudisili and Morris W. Firebaugh. Pp. xii+527. Oxford University: London, New York and Toronto, August 1975.) Boards £6.85; paper £4.00.

THE objective of the book is to provide clear and concise information on the arguments in America for assuring energy supplies, while not conflicting too much with environmentalists who oppose some of the methods. To produce what the authors believe to be a balanced and representative analysis in areas of controversy, they have made a selection of articles from the recent literature illustrating the energy and environmental dilemmas—reviewing the prospects of fossil and nuclear fuels as energy sources, considering alternative energy possibilities and finally ideas on energy policy for the future.

In each of five chapters the layout is similar—unusual but interesting. Each begins a group of photographs (generally clear and relevant to the subject matter of the papers in the chapter) and each is introduced by a few pages of synopses or commentary.

Glenn T. Seaborg, ex-US Atomic Energy Commission but now in his haven at Berkeley, Californa, repeatedly refers in his foreword to the "readings" in the book. And so the individual papers are, many of them original gems of authority. The book can be picked up and opened almost at random to find a self-sufficient specialist view on some subject of relevance in today's energy world. The 527-page tome, therefore, is really a short daily ration of "sifting and winnowing" to reach an intelligent informed opinion on energy politics assisted by the experts.

Chauncey Starr sets the pace with the "Realities of the Energy Crisis". and M. King Hubbert continues with a "Survey of World Energy Resources". The "Case for Nuclear Energy" is given by B. I. Spinrad followed by half a dozen pages nibbling away at topics like "Reactor Safety," "Disposal of Nuclear Wastes", and "Dilemma on Fission Energy and Pugwash warnings".

The book is rich in clear diagrams and useful tables, indeed worth at least the paperback price for the 24 in the Hubbert paper alone—more than one per page. There are plenty of references given in most of the 40 papers, and, as if that is not enough to keep one going into the small hours there is a suggested reading list at the ends of chapters.

Seaborg opens the debate with worry about a resource crisis (certain metals, food, water, energy) and wonders whether there is sufficient concern for the future. Ruedisili and Firebaugh provide plenty of thought-provoking material and cleverly end the book with Seaborg's answer to his own question on ideas for a "Recycle Society". Scrap would become secondary material but new material would be used rarely and only for top-up of the stock in use. Design for non-obsolescence, communications instead of transport, avoidance of urban sprawl and improvement of the energy situation could all contribute to a better life twenty years from now; that's not far away, he warns, and we had better get moving.

G. R. Bainbridge

Clarifying nuclear structure

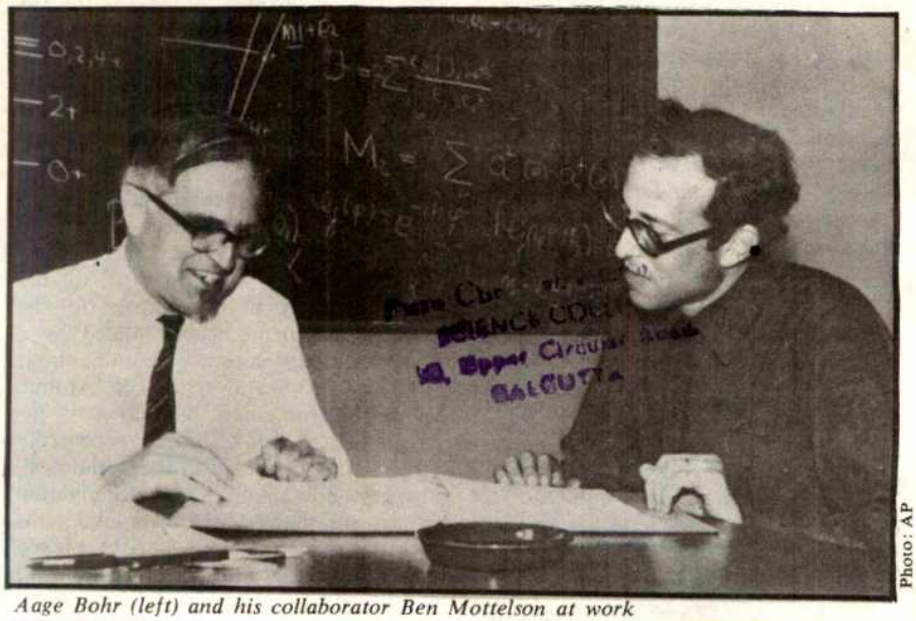

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Photo: AP

Aage Bohr (left) and his collaborator Ben Mottelson at work

Nuclear Structure. Volume 2 : Nuclear Deformations. By Aage Bohr and Ben R. Mottelson. Pp. xiii+748. (Benjamin: Reading, Massachusetts, November 1975.) $37.50.

FOR more than twenty years Bohr and Mottelson have devoted themselves to studies of nuclear structure, and have established in Copenhagen one of the world's leading centres for theoretical physics. Together with Professor James Rainwater, they have recently been awarded the 1975 Nobel Prize for physics in recognition of this work. Their seminars and lectures have gradually developed into a massive treatise of three volumes, of which this is the second. The first volume, on nuclear single particle motion, was published in 1969, and the third, on nucleonic correlations, is in preparation.

The central theme is the interplay of the independent particle and the collective aspects of nuclear structure, and the way this shows itself in the vibrational and rotational spectra of nuclei. The first volume covered the independent particle aspects in detail, and this volume is devoted to nuclear deformations, with chapters on rotational spectra, one-particle motion in non-spherical nuclei and on vibrational spectra.

The first of these discusses in turn the symmetry operations for rotating nuclei, the energy spectra and intensity relations for axially symmetric nuclei, the coupling between rotational and intrinsic motion for axially symmetric nuclei, and rotational spectra for systems without axial symmetry, and concludes with an appendix on the particle–rotor model.

The next chapter brings out the connection with the single-particle motions of the first volume by showing what happens to the motion when the potential is deformed. Successive sections are devoted to the stationary states and wave functions of particle motion in a spheroidal potential, and the classification of odd-A spectra and moments and transitions. This is followed by an appendix on scattering by nonspherical systems.

The last chapter, occupying half the book, is devoted to vibrati spectra. There is a full account of quantal theory of harmonic vibrati the normal modes of vibration and sum rules for multipole oscill strength. Sections follow on parti vibration coupling and anharmoni in vibrational motion; there are appendices on the liquid drop mode vibrations and rotations and on five-dimensional quadrupole oscill

The level of presentation is such it can be understood by a gr student, and yet it is so compre that most aspects of nuclear co motion are covered. In a book kind there is a danger that th is overwhelmed by a mass o and fails to see the essential the structure underlying the spe applications. Bohr and Mott attempt to avoid this by dividin book into three levels: text, illustr examples and appendices. The contains the basic theory, present as straightforward and unclutter manner as possible, and its applic to analyse the structure of part nuclei is relegated to the second The appendices contain mathematical derivations and mulae. On the whole this divisi successful in clarifying the pre tion.

As would be expected, the treat is thorough and detailed throu so that it will remain an indisp work of reference for many y come.

P. E. Ho

Quantum theory

Introduction to Axiomatic Quantum Field Theory. (Mathematical Physics Monograph Series, Volume 18.) By N. N. Bogolubov, A. A. Logunov and I. T. Todorov. Pp. xxvi+708. (Benjamin : Reading, Massachusetts, and London, July 1975.) Cloth $32.50.

THE publishers of this book are too optimistic when they say "it requires little preliminary knowledge of either mathematics or quantum field theory", and I prefer the authors' own assessment that "it would be useful to have an elementary knowledge of quantum field theory" and some undergraduate mathematics. It is a more complete treatise than *General Theory of Quantised Fields* by R. Jost (*Am. math. Soc.*, 1965) or *PCT, Spin and Statistics, and All That* by R. F. Streater and A. S. Wightman (Benjamin, 1964), and is naturally more up to date. It follows the same general lines, but with more detail in places and some topics are taken further. The inclusion of more group theory, wave equations and second quantisation certainly fills a gap the earlier books. Scattering theor better covered, and provides a logic introduction to the S-functio approach of Bogoliubov and to th perturbation expansion of Feynm and Dyson. This section ends wit brief account of renormalisa theory. There follows an accoun the general results of the the usually in great detail but occasion avoiding overlap with the other bo by referring to them. Infinite-c ponent fields and parastatistics among new topics. The book e with a very brief account of the algebra approach, and the prog of constructive field theory up to 1

The bibliography is quite large contains very useful references Russian work on this subject. book, being typed and photocopie rather bulky. It is well transl authoritative and suitable as a panion and guide for postgrad students in physics or mathem studying relativistic quantum m anics, and for other research wo seeking a way in to this difficult ject.

R. F. Str

cs and Chemistry of Surfaces.
'acques Oudar. Pp xii+130
<ie Glasgow and London, July
) £6 55

ESSOR Oudar's book aims to
le an introduction to two aspects
rface chemistry. The first sec-
is devoted solely to chemisorp-
of gases on metals, the rest of
ook concerns the oxidation of
s Clearly with less than 120
of script and that divided into
n chapters, no single topic can
scussed in any detail. Thus the
provides a cursory view of a
, which has undergone major
lopment in the last decade. The
igth of the book is that it has
its descriptions of modern tech-
es and recent results in the con-
of the older approaches to the
ect.

s a textbook it is too short to be
alue as a source of reference or
xtension of lecture notes It is,
ever, easy to read and very well
rated There are occasional
rs in the transcription and some
he concepts presented are not
ersally held. It does provide, how-
, a valuable overview of the
ect but it is rather expensive for
length **A. E. Lee**

Early Development of Mammals
e Second Symposium of the British
iety for Developmental Biology)
ted by M. Balls and A. E. Wild.
vii+410. (Cambridge University
ss: Cambridge and London, 1975)
00; $49 50

is is an excellent book, covering
ny of the current lines of research
mammalian embryology The 23
pters include culture and storage
thods, fertilisation, partheno-
esis, differentiation, expression of
nes and antigens, induction,
velopment of the lymphoid and
emopoietic systems, teratomas and
ratocarcinomas, sex reversal,
hymia and cell death Inevitably, the
ouse holds the centre of the stage,
it other mammals make brief
pearances from time to time.

The 30 contributors are all actively
gaged in research in mammalian
velopment and write with authority.
ost give a short review of previous
rk in their particular field followed
an account of recent experiments
general, the standard of present-
on is high The style is concise but
dable, the illustrations and index
e adequate and there is the mini-
um of repetition between chapters.
n occasional paragraph is muddled or incomprehensible and would have benefited from tougher editing, but such blemishes are few and become insignificant when set beside all that is good. The British Society for Developmental Biology, and particularly the two editors, have clearly had a major task in bringing together so much in one book and they are to be congratulated on the result

The book will be of great value to all research workers on mammalian development and to many other biologists who want to know where the subject has got to. Its wide coverage and attractive presentation should also make it stimulating reading for teachers and students of undergraduate courses in developmental biology Unfortunately, many potential readers will be put off by the price from buying the book for themselves **D. A. T. New**

Books brief

Chemistry of Viruses. Second Edition. (Springer Study Edition.) By C. A. Knight Pp. x+325. (Springer: Berlin, Wien and New York, 1975.) DM48; $20.70.

KNIGHT has set out to produce a compendium of chemical information covering the major classes of viruses. The result is an impressive collection of data dealing almost exclusively with the virus particle and presented in a style somewhere between a handbook and a textbook Purification and especially composition are considered in great depth, often including precise experimental details, and clearly reflect the author's wide experience and knowledge of the subject Particularly useful is the treatment of the four virion components (protein, nucleic acid, lipid and carbohydrate) in terms of methods of purification, methods of analysis and function. Unfortunately there is no mention of the agarose gel electrophoresis and restriction endonuclease cleavage techniques which are currently much in use for nucleic acid analysis. Also absent is any reference to the cohesive, repetitious and inverted repetitious base sequences present at the ends of many types of viral DNA genome. The interactions between virus particles and chemical agents which lead to inactivation and mutation are discussed in a clear and reasonably comprehensive manner albeit in somewhat less depth than earlier chapters. The final chapter collects together a set of topics which make curious bedfellows, for example, virus reproduction, genetic recombination, viroids, reconstitution, cell-free synthesis and origin of viruses. They are discussed very superficially, much like an afterthought, and could well have been omitted or included in a general introduction. But this is otherwise a minor criticism of a worthwhile book which will serve as a very useful source of information on most aspects of the virion.

D. A. Ritchie

Equilibrium and Non-equilibrium Statistical Mechanics. By Radu Balescu. Pp. xiv+742. (Wiley-Interscience: New York and London, 1975.) £16.20.

STATISTICAL mechanics is a fascinating and confusing subject: it is tremendously successful in practice, but its ultimate justification has proved surprisingly difficult to establish. And although there has been much recent work on foundations, it is forbiddingly difficult for the average physicist to understand. This book is the one to read in order to remove one's misconceptions and in order to achieve an understanding of the subject.

The style and presentation are admirably clear, and there are many enjoyable asides and perceptive comments. Among the applications selected for discussion, plasmas and classical gases predominate, and there is an excellent account of modern work on critical phenomena, including Wilson's theory. In the non-equilibrium section the emphasis is on kinetic equations and their justification by the methods of the 'Brussels school' of which the author is a distinguished member. There is a delightful appendix giving a nontechnical summary of recent work on the ergodic problem.

Obviously, in such a vast subject every author has his own preferences, and for my own taste there is not enough emphasis on specific quantum phenomena: quantum theory tends to be treated as a correction to classical physics, and some of the most exciting applications of statistical mechanics, to photons and superfluids, are barely mentioned But within its self-imposed limits this book is certainly a very valuable addition to the literature and deserves to be widely studied. **E. H. Sondheimer**

obituary

Dr J. F. Heremans, Professor of Internal Medicine at the Catholic University of Louvain, died on October 29, aged 48. He received his medical degree with highest distinction, at Louvain in 1952, and after specialising in internal medicine, worked with Professor Waldenström in Malmö (1957–1958), and with Professor Kunkel at the Rockefeller Institute (1959). He became Professor at Louvain in 1965, and was one of the founder members of the newly created International Institute for Cellular and Molecular Pathology in Brussels. His expertise was recognised by the award of several national scientific prizes, and he was a member of many learned societies throughout the world, notably the European Society for Clinical Investigation, of which he was elected President in 1971.

While interested in all aspects of experimental medicine, his major research interest was immunoglobulins. The most significant of his discoveries is this field was the isolation and characterisation of IgA

Having established its chemical similarity to the other two major types of antibody molecule, he proposed the term 'immunoglobin' as descriptive of all three, and afterwards played a large part in the development of our present unified concept of antibody structure and function.

Professor Heremans was always aware of the need for accurate quantitation of proteins, whether in the research or clinical fields, and it was under his direction that the Mancini Radial Immunodiffusion Technique was developed. More recently, a variety of automated immunoassay techniques suitable for such diverse applications as plasma protein screening and drug and hormone measurements have originated in his laboratory.

His encyclopaedic knowledge
tein structure and function
illustrated by the compendious 'I
lar Biology of Human Proteins',
before his 40th birthday, in co
tion with Professor H. E. S
Now a standard reference work
demonstrates his extraordinary
tic ability; he was completely fl
five European languages, an
versant with several others.

Professor Heremans was a
very wide-ranging interests; he
pianist of considerable talent
keen amateur lepidopterist. He
tained a lively interest in the
thise in his laboratories, to wl
was always available to give adv
leaves a wife and five childı
whom, as well as by his scient
leagues and friends; he will be
missed.

John Peters

announcements

International meetings

January 8–9, **Molecular developmental biology,** Glasgow (Department of Zology, University of Glasgow, Scotland).

February 18, **Materials microanalysis with less than 1,000 Å spatial resolution,** Churchill College, Cambridge (The meetings secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20 9LW, UK).

February 20, **Great women in science,** Boston (American Association for the Advancement of Science, 1176 Massachusetts Avenue, NW, Washington DC, 20036, USA).

February 25, **Professional metallurgists in Europe,** Imperial College, London (The meetings secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20 9LW, UK).

Reports and publications

Other Countries

United States Department of the Interior Geological Survey Water-Supply Paper 2133 Surface Water Supply of the United States, 1966–70 Part 12 Pacific Slope Basins in Washington. Vol 2: Upper Columbia River Basin Pp. viii+674 Water-Supply Paper 2171 · Ground-Water Levels in the United States, 1969-73. Southeastern States Pp. v+250. (Washington, DC Government Printing Office, 1975) [311

United States Department of Agriculture Index-Catalogue of Medical and Veterinary Zoology Supplement 19, Part 5: Parasite-Subject Catalogue—Arthropoda and Miscellaneous Phyla. By Jane D. Rayburn, Martha Walker Hood, Margie D. Kirby and Judith H. Shaw Pp iv+403 (Washington, DC: US Government Printing Office, 1975) $4.60 [411

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